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ECBC-TR-463

DETECTION AND IDENTIFICATION OF VIRUSES USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

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GEO-CENTERS, INC. - GUNPOWDER BRANCH

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14. ABSTRACT
Detection and identification of viruses present a particularly difficult challenge. In this report, we describe how a newly invented device detects and identifies viruses by measuring their physical characteristics (size) and breakdown products. The report starts by briefly describing the Integrated Virus Detection System (IVDS) and its components. It also describes how we characterize several known viruses. In addition, this report shows how the IVDS can be used to isolate and characterize viruses from complex media (e.g., drinking water, blood plasma, and sea water). Finally, we suggest some applications where the IVDS could be useful.

15. SUBJECT TERMS

Virus detection	Differential mobility analyzer	Homeland defense	Particles condensation counter
Biological agents	Bio-terrorism	MS-2	Biological warfare
AIDS research	Medical research		

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PREFACE

The work described in this report was authorized under Contract No. DAAD13-01-C-0016. The work was started in March 2001 and completed in March 2003.

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CONTENTS

FIGURES

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DETECTION AND IDENTIFICATION OF VIRUSES
USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

1. INTRODUCTION

Viruses are considered to be among the smallest living particles known to man. Figure 1 illustrates the comparative size between the different types of particles. Because of their small size, viruses are extremely difficult to detect and characterize. Since viruses can cause many diseases in humans and can also affect crops and domestic animals they are of great concern to the public health authorities, and can have a major economic impact by causing plant and animal diseases.

Presently, detection and identification of viruses are complex and expensive biochemical processes that require great expertise.[1] Even new methods, such as "a new silicon chip that harnesses emerging technology at the nano scale, that will allow the detection of viruses faster, and more accurately, than ever before",[2] rely on the newest technology, and are essentially biochemical methods. The detection process is particularly complex and lengthy when an unknown virus "hits the street". This was illustrated in recent years by the length of time it took to discover and identify the HIV virus.

Recently, the U.S.Edgewood Chemical Biological Center (ECBC) developed a new system by which viruses are detected and identified by physical rather than biochemical means. This system, the Integrated Virus Detection System (IVDS), relies on the fact that different viruses have different sizes. The system isolates the virus particles from the extraneous material in which they are collected, separate them according to their sizes using, a Differential Mobility Analyzer (DMA), and determine their concentrations with a Condensation Particles Counter. The system allows quick screening of many samples at a low cost.

Using the IVDS we developed detailed procedures to isolate and characterize close to 30 different viruses. In this report we will first describe briefly the principles of operation of the IVDS. We will then describe in detail the procedures by which we isolated and characterized each of the viruses. As new viruses are added to this list we will publish annual annexes to this report describing the procedures for these new viruses.

2. DESCRIPTION OF THE IVDS

The IVDS is shown in Figure 2. It is described in detail in ECBC-TR-018.[3] For the sake of completion we will briefly describe here its principles of operation and its various components. For more details, the reader is referred to the ECBC-TR-018[3] and other publications[4,5] by Wick and others.

The IVDS is a modular system built around four principle components:

a. purifier/concentrator

b. Electrospray injection
c. a size analyzer comprised of DMA
d. a condensation particles counter, which is used to determine the concentration

The IVDS is designed as a modular system to allow upgrades of different components as they are being developed and improved.

2.1 The Purifier/Concentrator.

The Ultra Filtration (UF) system is shown in Figure 3. As its name implies, the UF system removes extraneous material from the sample and concentrates the virus like particles to a point that they can be detected.

The UF system consists of a fiber-based tangential flow filtration system where the more coarse contaminations are removed and the sample volume is reduced to <1 ml. The initial sample volume can range from few milliliters to several liters. A schematic diagram of this subsystem is shown in Figure 4. The most important component of this stage is comprised of a bundle of hollow fibers whose walls and made of permeable membrane with wide range of pore size. An example of such individual fiber is shown in Figure 5. The sample is pumped through the semi-permeable fiber, and the filtrate is forced through the fiber's walls by the pressure differentials. The sweeping action of the sample stream prevents clogging of the fibers. Fibers with pore sizes from $4\mu m$ diameter to 100,000 Da are available.

2.2 Electrospray Injection.

The sample solution is stored in a cone-shaped vial, enclosed in a cylindrical pressure chamber. The chamber accommodates the inlet capillary and a platinum high-voltage wire, both of which are immersed in the solution. Maintaining a differential pressure causes the solution to be pushed through the capillary. The fluid containing the particles exits the capillary and is sprayed through a strong electric field that causes it to form a cone and break into small charged droplets. To prevent corona discharge the cone is surrounded by CO_2. As the droplets evaporate and dry out they form a plume of particles that comprise of what ever virus like particles (or other large molecules) that the original sample contained and solute salt particles. If the concentration of the virus like particles is not too high, each particle will contain a single virus.

2.3 Differential Mobility Analyzer (DMA).

A differential mobility analyzer separates particles according to their mobility in an electric field. The electrical mobility of a particle is a function of its size and the number of charges in contains. For a more complete theoretical background the reader is referred to any good text book on aerosol physics. Fuch's book is an excellent example.[6] For particles in the size range of few nano-meters the electric charge that a particle will acquires is limited to a single elemental charge. Thus, the mobility of the charged particles in an electric field will be limited to their size only.

The plume of the poly disperse particles enter the DMA as is shown schematically in Figure 6. The DMA comprises of a cylinder with a central rod. A controlled DC potential differential (0-10,000 VDC) is applied between the cylinder and the central rod. By controlling this potential, only particles with very narrow electrical mobility (size) are allowed to enter the opening slit at the bottom of the cylinder and enter the particles counter. Figure 7 shows the output of the IVDS when injected with the mixture of MS-2 virus (23.3 nm diameters) and calibrated 70 nm diameter Poly Styrene beads. Figure 8 illustrate the resolution capabilities of the IVDS. A mixture of Rice Yellow Mosaic virus and MS-2 was prepared in the laboratory and analyzed. The peaks of these two viruses (4 nm apart) are clearly separated.

2.4 Condensation Particle Counter.

The particle counter is based on the fact that in order for a vapor to condense and form droplets in the absence of any particles the vapors need to reach a high degree of supersaturation.* Water vapors, for example, need to reach supersaturating of 800% before spontaneous (also known as homogeneous) nucleation occurs. In the presence of small particles, these particles serve as nucleation (condensation) centers and condensation occurs at lower supersaturating, which is a function of the particles' size. It can be shown that all the nucleating particles grow to droplets of identical size (which depends on the availability of vapors). The number of particles than is identical to the number of droplets forms. This number can be deduced by measuring the opacity (or light transmission) in the volume containing the particles.

In the IVDS, the condensing vapor is n-butanol. The aerosols laden air from the DMA passes through a heated chamber (39 ^{o}C) over a pool of liquid butanol. The butanol saturated air passes into another chamber held at lower temperature (10 ^{o}C) where it become supersaturated and the butanol vapors condense on the particles that are present to form a "fog." The density of this "fog" is a measure of the number of particles present and is determined by measuring the attenuation of light. The supersaturation that can be achieved, and hence the smallest particle that can be activated, is a function of the temperature differential between the warmer and cooler chambers. In the IVDS the supersaturation that is achieved is such that particles smaller than 10 nm can be activated and counted.

3. PROCEDURES FOR SAMPLE ANALYSIS

3.1 General.

The process cycle/logic for analysis of a sample for viruses are described in Figure 9. As can be seen, the analysis process depends on the "quality" of the sample. If the sample is clean and concentrated enough, the process is straight thorough. However, if the sample contains impurities and/or is not concentrated enough the sample goes through purification and concentration processes to make it suitable for analysis.

* Super saturation is defined as the ratio of the actual vapor pressure P of the condensing vapor to P_0, which is the saturation vapor pressure over the flat surface of the liquid.

Figure 10 is an example of results obtained from a "raw" MS-2 sample. The wide, long tail peak is indicative of growth media. After passing the sample through the UF system, a nice peak of MS-2 is seen at 23.3 nm (Figure 11).

Figure 12 shows the IVDS's output when injected with a sample of GD7 Picornavirus received from Charles River Labs (WSV 062899). The large symmetrical peak is characteristic to large concentration of salt. After diluting the sample 1:100 with 20 mM ammonium acetate, reconcentrating it by the UF subsystem and rerunning with multiple scan accumulation, we clearly see a peak at 31.1 nm (Figure 13). Full scale expansion also shows a large peak at 15.1 nm (Figure 14), which is possibly protein or cellular debris.

A virus has several components of different sizes that can aid in its identification. Figure 15 shows a scan of an Alphavirus. The intact virus has a peak at 70 nm. Doublets and triplets are seen at about 95 nm and 107 nm, respectively. In addition, we see large signals at 49 nm and 36 nm that are likely the stripped outer protein and the core material, respectively. Another example can be seen in Figure 16 which shows the intact MS-2 virus (27 nm) and its building blocks-the RNA core (18 nm) and the protein coat (13 nm).

3.2 Analysis and Results of Specific Viruses.

In this section we will describe the results of analysis of specific viruses. Note that various properties of the viruses are given in the Appendix and in specific references mentioned throughout the text.

3.2.1 GD7 Virus.

The GD7 Virus, also known as Picornavirus-icosahedral is a murine encephalomylitis virus. It was received from Charles River laboratory (Wilmington, MA). Its approximate size is 22-30 nm.[7] Other properties of the virus are also given in reference 7. The virus was obtained in solution containing salts and growth media. A scan of the original solution is shown in Figure 12, where the large peak from the salt masks any virus peak. The virus solution was diluted at a ratio 1:100 with 20 mM solution of ammonium acetate. A multiple accumulation scan of the diluted solution (Figure 13) shows a clear peak at 31.1 nm and companion peaks at 25 nm and 22.5 nm, which are likely protein breakdown products. Full expansion of the peak also shows a large peak at 15.1 nm and 9 nm (Figure 14). These are likely characteristic protein breakdown products or cellular debris.

3.2.2 Kilham Rat Virus.[8]

The virus (lot 091991) was received from Charles River Laboratory in a salt solution. Additional information about this virus can be found in reference 8. A run of the neat solution (Figure 17) shows a large symmetrical peak that is likely the salt peak. After diluting the solution 1:100 with 20 mM ammonium acetate, UF with 100K Da filter and rerunning with multiple scan accumulation, we got the results shown in Figure 18. The main peak of the KRV virus appears at 22.5 nm with companion peaks (at much lower concentrations) at 34.6 nm, 49.6 nm, and 71 nm. These are likely particles that contain 2 or more viruses each, or contamination from other virus particles. A large peak at 13.6 nm is likely a protein break down product or cellular material that was not removed by filtration.

3.2.3 MAD-K87 Adenovirus.[9]

The MAD-K87 Adenovirus was received from Charles River Labs (WSV 072098) in a salt solution. A run of the neat solution shows a main virus peak at 82 nm and a companion peak (at 57.3 nm), probably of a protein break down product (Figure 19). After 1:100 dilution with 20 mM ammonium acetate to remove the salt peak the analysis shows a peak at 25.9 nm (Figure 20), and a full scale visualization (shown in Figure 21) shows additional peaks at 15.1 nm and 10.9 nm, which are probably protein breakdown products.

3.2.4 Murine Hepatitis Virus (MHV) or Coronaviridae.[10]

The Murine hepatitis virus (MHV, lot 120998) known also as Coronaviridae is an enveloped virus. The virus was received from Charles River Laboratory in salt solution. Figure 22 shows the enveloped virus at 73.7 nm and the uncoated at 51. 4 nm. The virus is unstable and breaks apart when diluted 1:20 with 20 mM ammonium acetate or 50 mM Potassium Phosphate (Figures 23 and 24, respectively). It is interesting to note that the break down products in both cases are different even though in both cases the pH was almost identical (7.1 and 7.2).

3.2.5 MVM Parvovirus.

This is a non-enveloped virus from Charles River Laboratory in salt solution. A scan of the neat solution (Figure 25) could not resolve the virus peak due to the large salt peak. Diluting the solution 1:100 in 20 mM ammonium acetate reveals the virus peak at 26.9 nm (Figure 26). Figure 27 shows protein break down products at 15.1 nm and 10.9 nm.

3.2.6 Reo-3 Reovirus.

The Reo-3 Reovirus was received from Charles River Laboratory in salt solution. A scan of the neat solution (Figure 28) shows the virus peak at 79.1 nm and a possible viral core at 53.3 nm. 1:100 dilution with 20 mM of ammonium acetate shows a possible breakdown peak at 24.1 nm (Figure 29) and 15.1 nm (Figure 30).

3.2.7 Sendai Rodent Virus.

A scan of the Sendai Rodent Virus diluted 1:20 with 20 mM ammonium acetate is shown in Figure 31. A major peak at 28.9 nm is evident. No break down fragment can be detected.

3.2.8 Summary IVDS Results.

The IVDS results are summarized below:

Virus Name	Virus diameter (Major peak - nm)	Protein break down products (nm)
MS-2	22.3	13, 18
GD-7 Picornavirus	31.1	9, 15.1, 22.5, 25
Kilham Rat Virus (KRV)	22.5	13.6
MAD-K87 Andenovirus	82	10.9, 15.1 25.9, 57.3
Murine Hepititis Virus (MHV) or Coronaviridae	Enveloped virus 73.7 Uncoated virus 51.4	Dilution in ammonium acetate: 23.3, 27.9 Dilution in potassium phosphate: 35.9, 40
MVM Parvovirus	26.9	10.9, 15.1
Reo-3 Reovirus	79.1	15.1, 24.1, 53.3
Sendai Rodent virus	28.9	

3.3 Analysis of Complex Media.

3.3.1 Detection of Viruses in Drinking Water.

Figures 32, 33, and 34 illustrate the ability of the IVDS to detect viruses in a "complex" media. In this case, 1 mL of mixed media MS-2 was mixed in 500 mL of drinking water and counted. As can be seen in Figure 33, no discernable MS-2 is observed. However, after the sample was processed through the UF subsystem and concentrated to a volume of 1.2 mL, the MS-2 peak is clearly visible (Figure 34).

3.3.2 Recovery of MS-2 from Spiked Blood Plasma.

Figure 35 shows the ability of the IVDS to detect viruses in a complex media such as blood plasma. Cynomolgus monkey Plasma was spiked with MS-2. The spiked solution was diluted 1:100 with 20mM ammonium acetate buffer solution, ultra-filtered through 100K Da fiber filter and scanned. The MS-2 peak at 22.3 nm is clearly visible. In addition there is a distinct peak at around 15 nm, which is likely a protein peak.

3.3.3 Analysis of Marine Water.

Sea water is known to be rich in biologically active material. Figure 36 illustrates IVDS scan of a sample of marine water. Four liters of water was collected at Rehobeth Beach, DL. The 4 L was concentrated to 40 mL and than to 3 mL by the UF subsystem. To reduce salt peak the solution was diluted 1:10 with 20 mm buffer solution of ammonium acetate and scanned by the IVDS. Figure 36 is an accumulation of 500 scans. Several unidentified peaks between 29 and 89 nm are clearly discernable as well as between 10 nm and 20 nm, which are likely proteins or cell fragments. Figure 36 also illustrates the possible complexity of the marine aerosols† and further emphasizes the need to better understand the biological background of the marine environment as part of a strategy to detect any possible bioterrorism attack from the sea.[11]

3.3.4 Analysis of Saliva.

Figure 37 shows an IVDS of human saliva. The saliva was mixed with distilled water, filtered through 100K Dalton fiber in the UF module and finally mixed with 10 mL of 20 mM ammonium acetate and scanned. No viruses can be observed. However, several peaks with sizes between 5 nm and 25 nm are observed. These likely represent different proteins.

3.3.5 Analysis of Environmental Air Sample.

An air sample was collected at the Edgewood area of Aberdeen Proving Ground using the Spincon.‡ The collected aqueous media was prefiltered through $0.22 \mu m$ filter, then passed through 100K Da fiber in the UF module of the IVDS, washed with 30 mL of 20 mM ammonium acetate and scanned. Figure 38 shows the scan results. Several peaks between 5nm and 70 nm are clearly observed.

4. POTENTIAL APPLICATIONS OF THE IVDS

The IVDS can have several applications in the medical and defense arenas, including homeland defense.

In the medical arena the IVDS can have applications in the clinical and research areas. As a research tool it can be used to detect the presence of unknown viruses. For example, when the AIDS epidemic started it took many years of research to discover that the culprit is an unknown (at the time) virus. It is very possible that the presence of this virus could have been found by the IVDS, if it would have existed at the time. In that case research for controlling the disease could have been started much earlier.

As a clinical tool AIDS again comes to mind. HIV positive patients who control their disease by medication need to undergo a blood test every few months in which the concentration of the virus is tested to insure that their medication regime is still effective. This test is fairly expensive and labor intensive. With the IVDS this test could be simplified and its cost brought down. In addition, it is known that periodically the HIV virus mutate and the medication regime needs to be changed. It is probable that the mutated virus either has different size and/or different breakdown product. The IVDS could possibly detect the mutated virus

† Biological materials are ejected into the air together with the sea salt particles as a result of the breaking waves.
‡ The Spincon is a wet cyclone particle collector/concentrator that collects respirable aerosols from the air into an aqueous media. It is manufactured by Sceptor Industries Inc. (Kansas City, MO).

early and allow the physician to start a new medication regime before the new virus causes the patient too much damage. Early detection of hepatitis is another potential clinical application for the IVDS. There are several (5) types of hepatitis viruses that are very different in size. Using the IVDS will allow early detection of the disease and initiation of treatment to minimize the effect on the patient and the transmissibility of the disease well before any symptoms show up.

Viruses can potentially be used as a biological weapon on the battlefield or as a terror agent. At the present time, we do not have any virus detection capabilities. Mating with an appropriate aerosol collector, the IVDS can fill this gap in our detection capabilities.

In areas, the medical field and the defense arena we need to characterize the known viruses. This requires concerted effort to characterize the size and breakdown products of all known viruses and to develop a data base that will include all relevant information. Similarly, we need to characterize the background atmosphere in term of viruses and virus like particles it contains naturally and how this natural background varies in time and space.

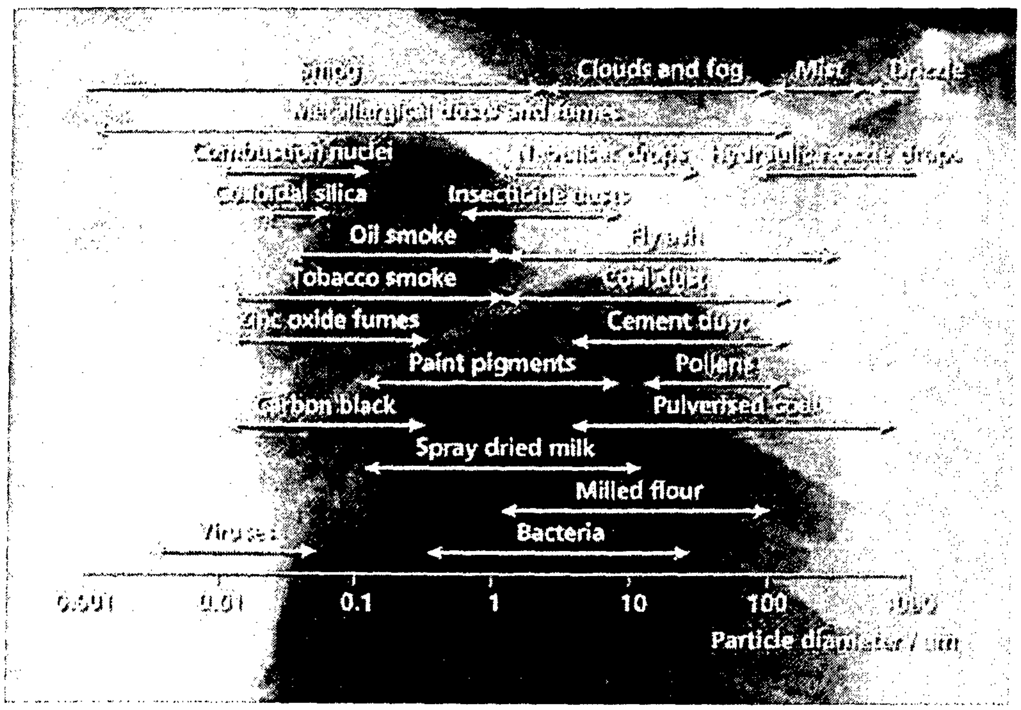

Figure 1: Comparing Sizes of Particles of Different Types

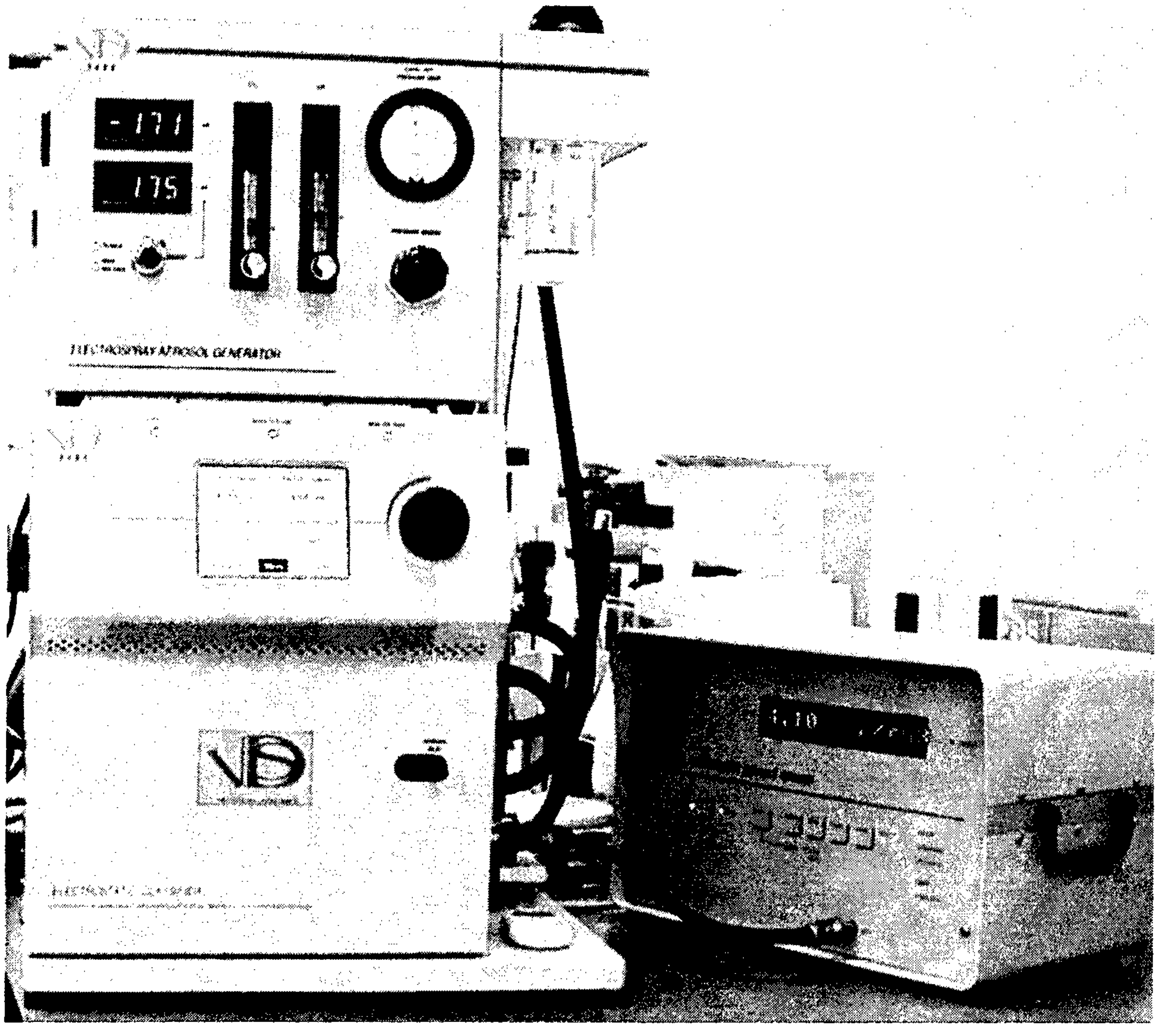

Figure 2: Picture of the IVDS

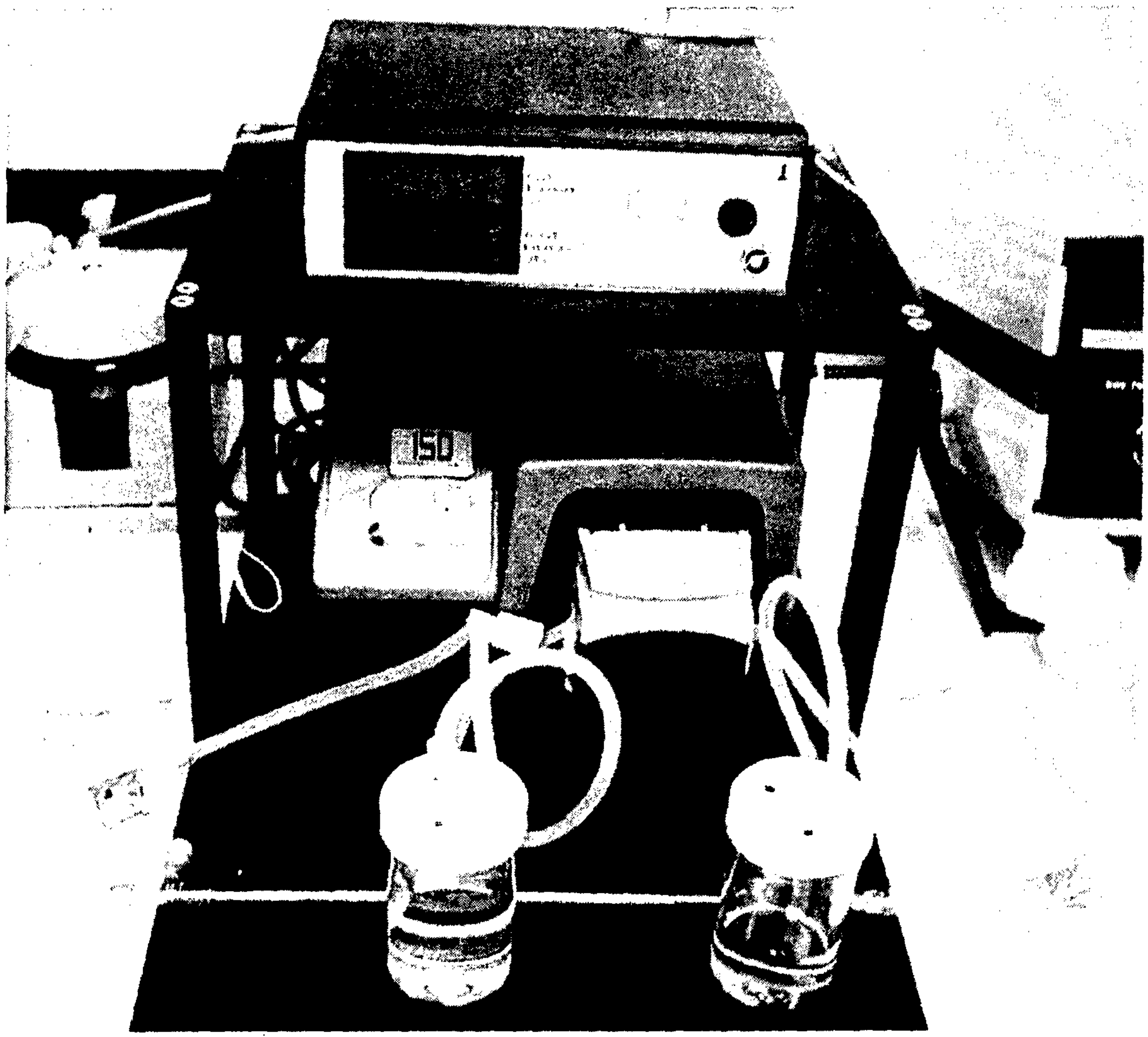

Figure 3: Filtration and Purification Subsystem

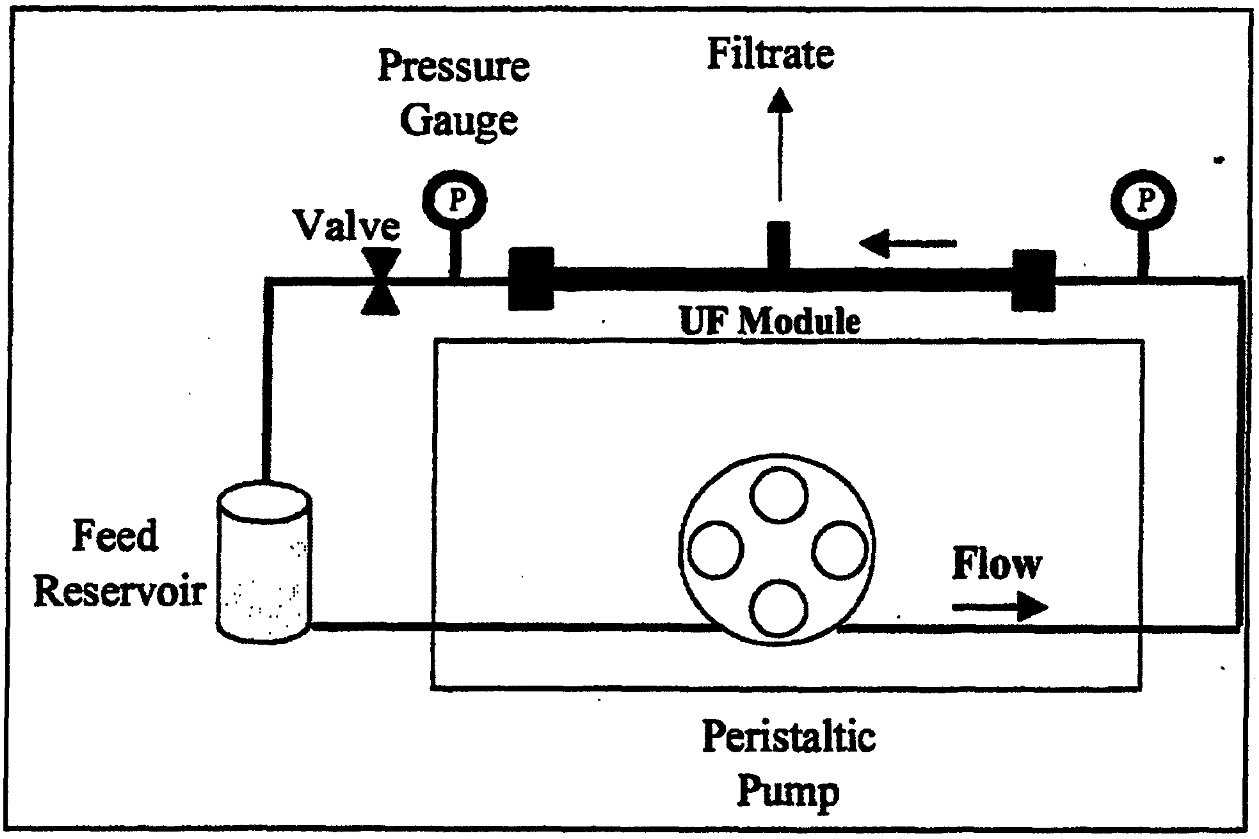

Figure 4: Cross Flow Ultra Filtration System

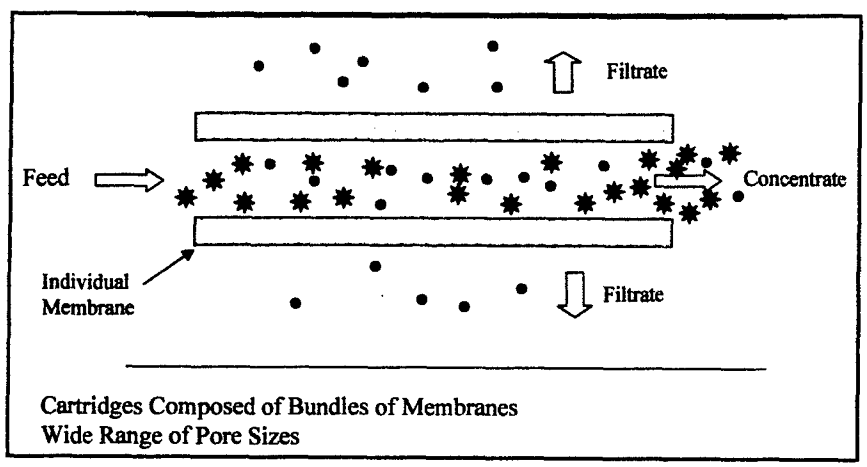

Figure 5: Individual Fiber of the Cross-Flow Filtration System

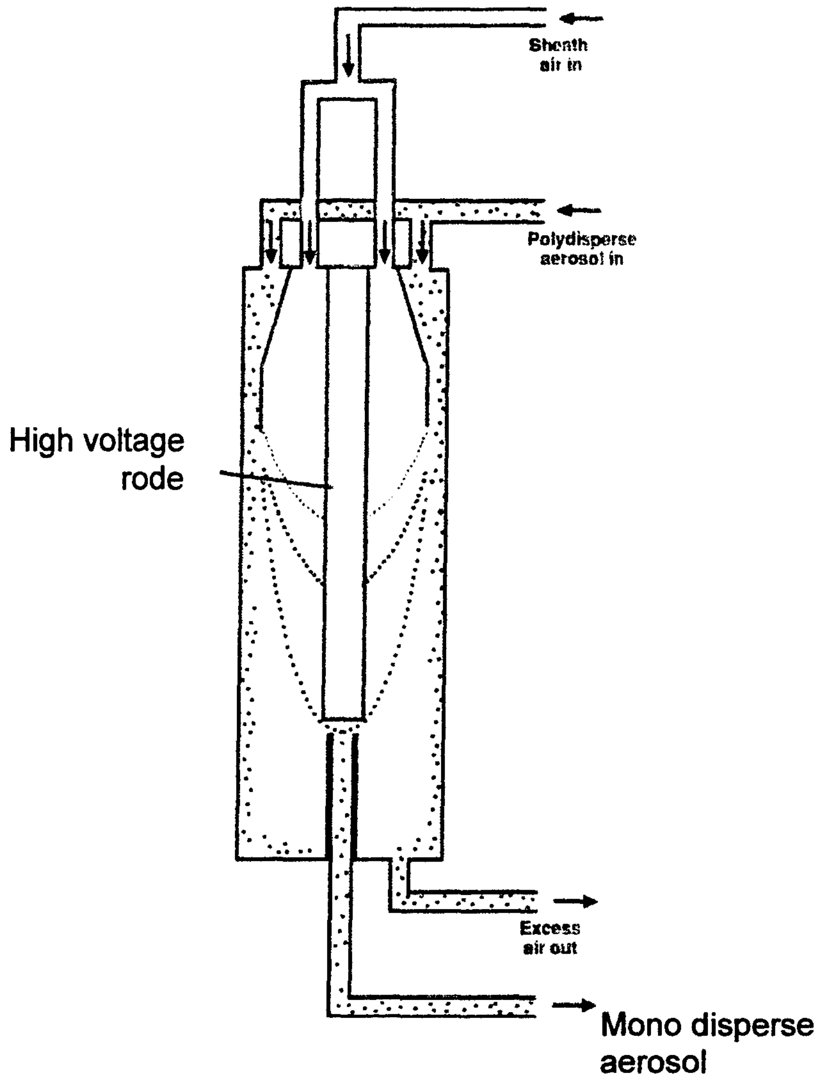

Figure 6: Differential Mobility Analyzer

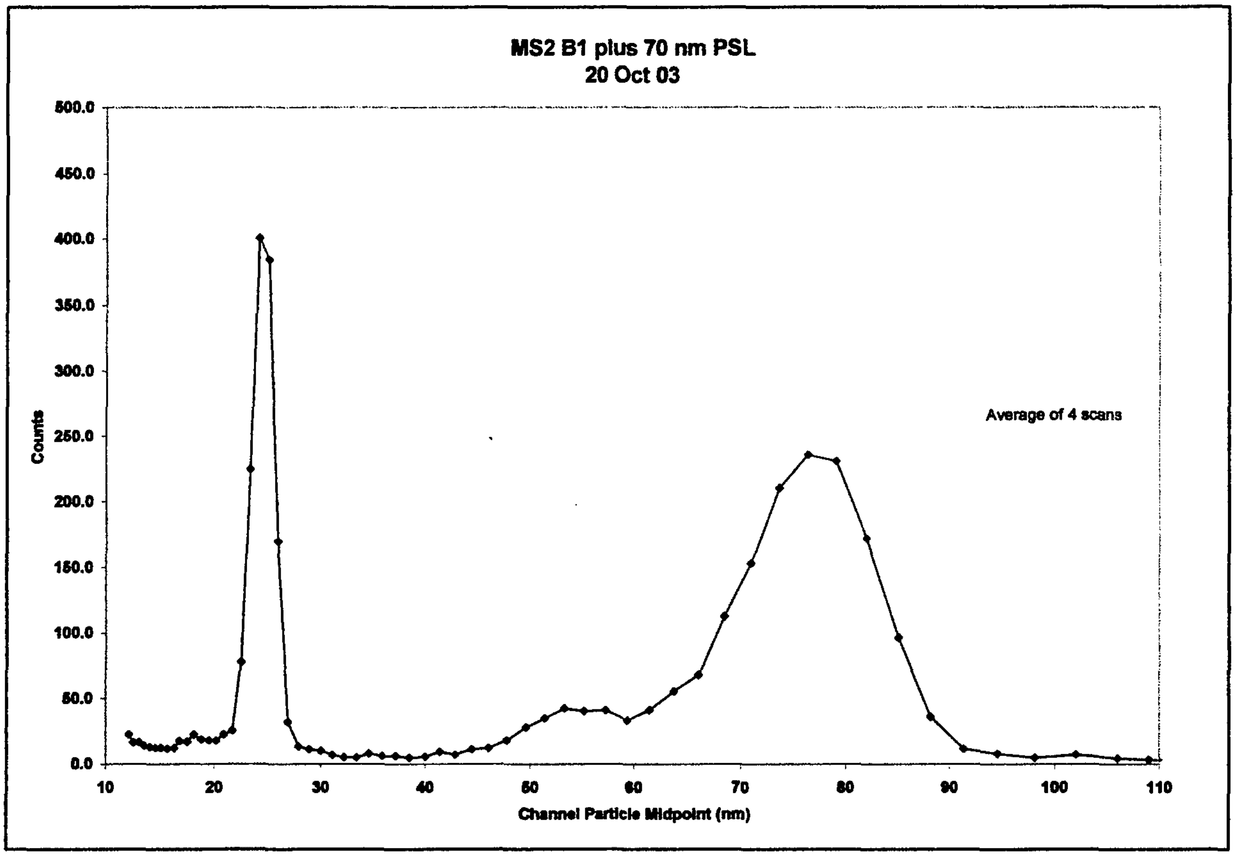

Figure 7: Mixture of MS-2 and 70 nm PSL Beads

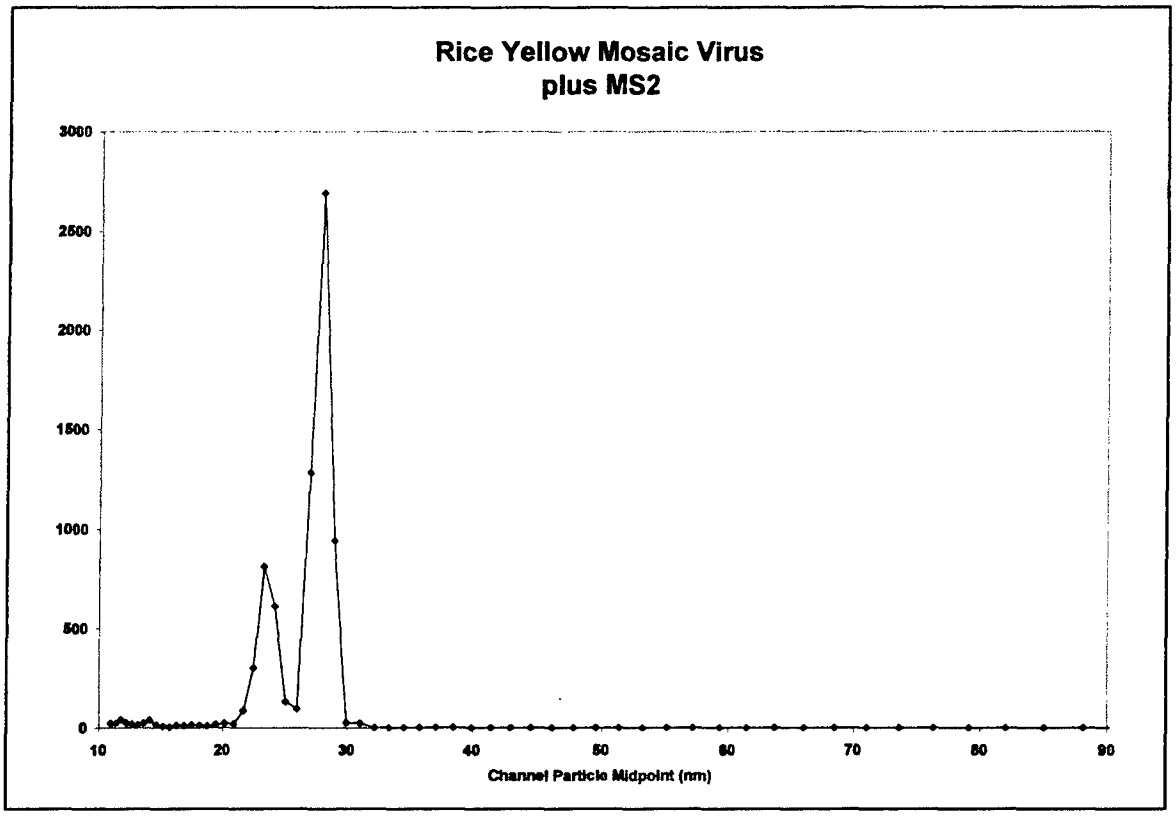

Figure 8: Illustration of the Size Resolution of the IVDS

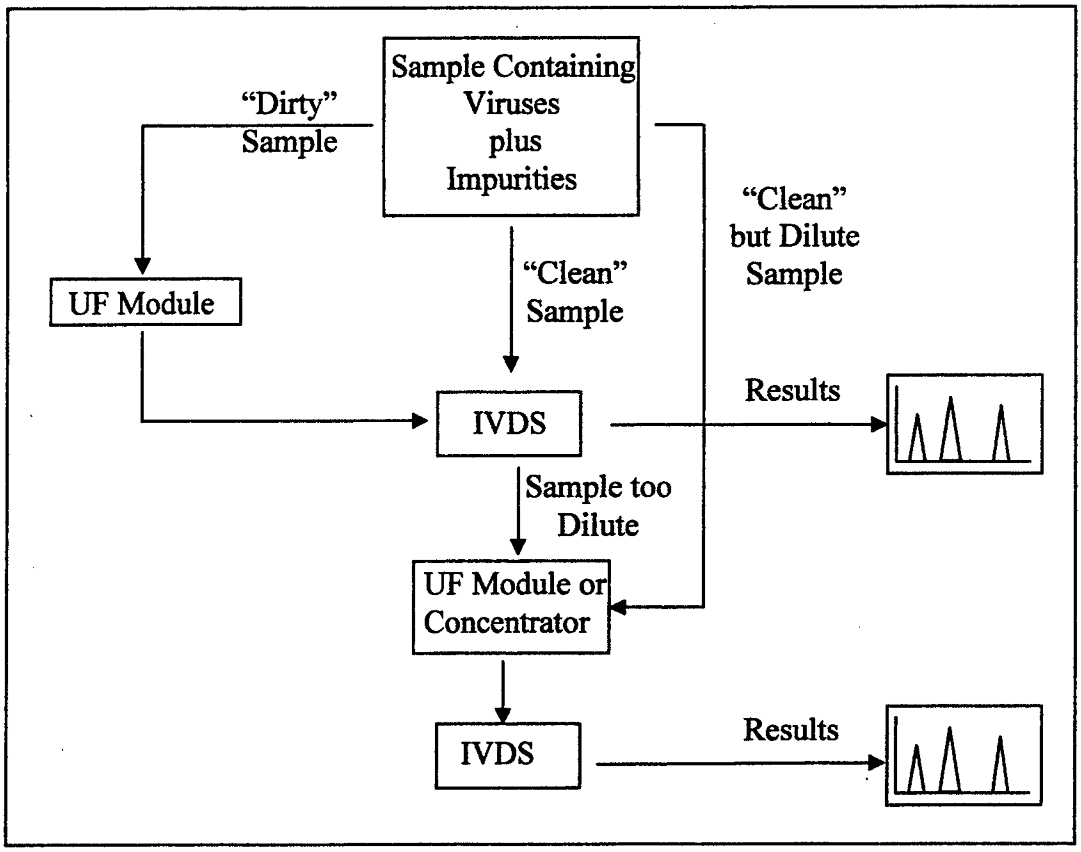

Figure 9: Schematic Diagram Describing the Logic Tree for Detecting Viruses

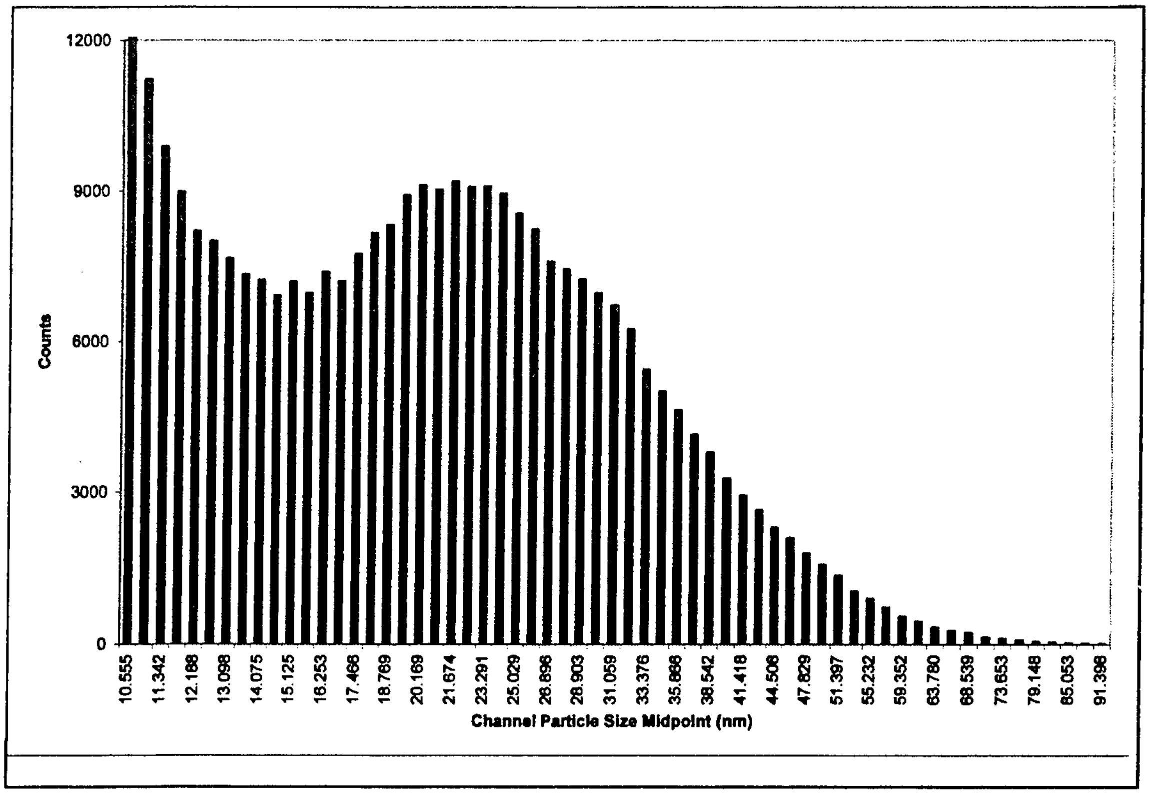

Figure 10: Example of a Raw, Unpurified MS-2 Sample

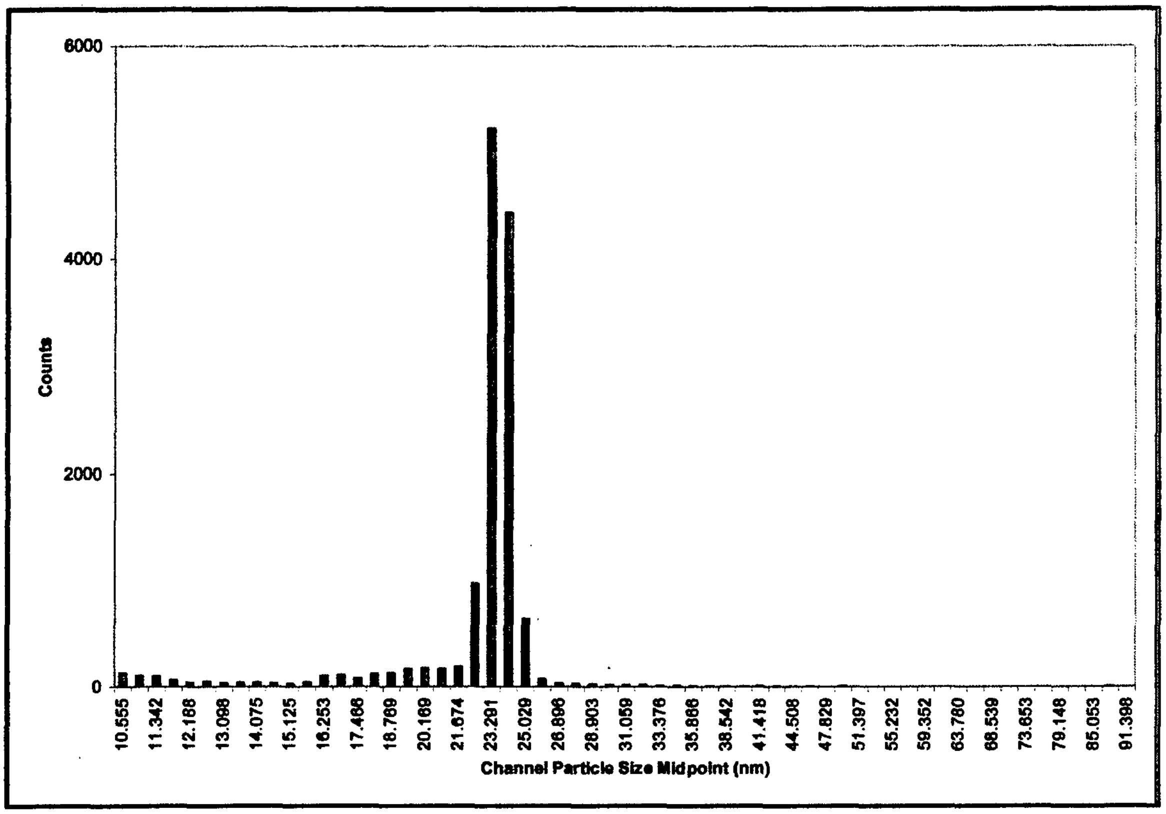

Figure 11: Sample of MS-2 after Purification

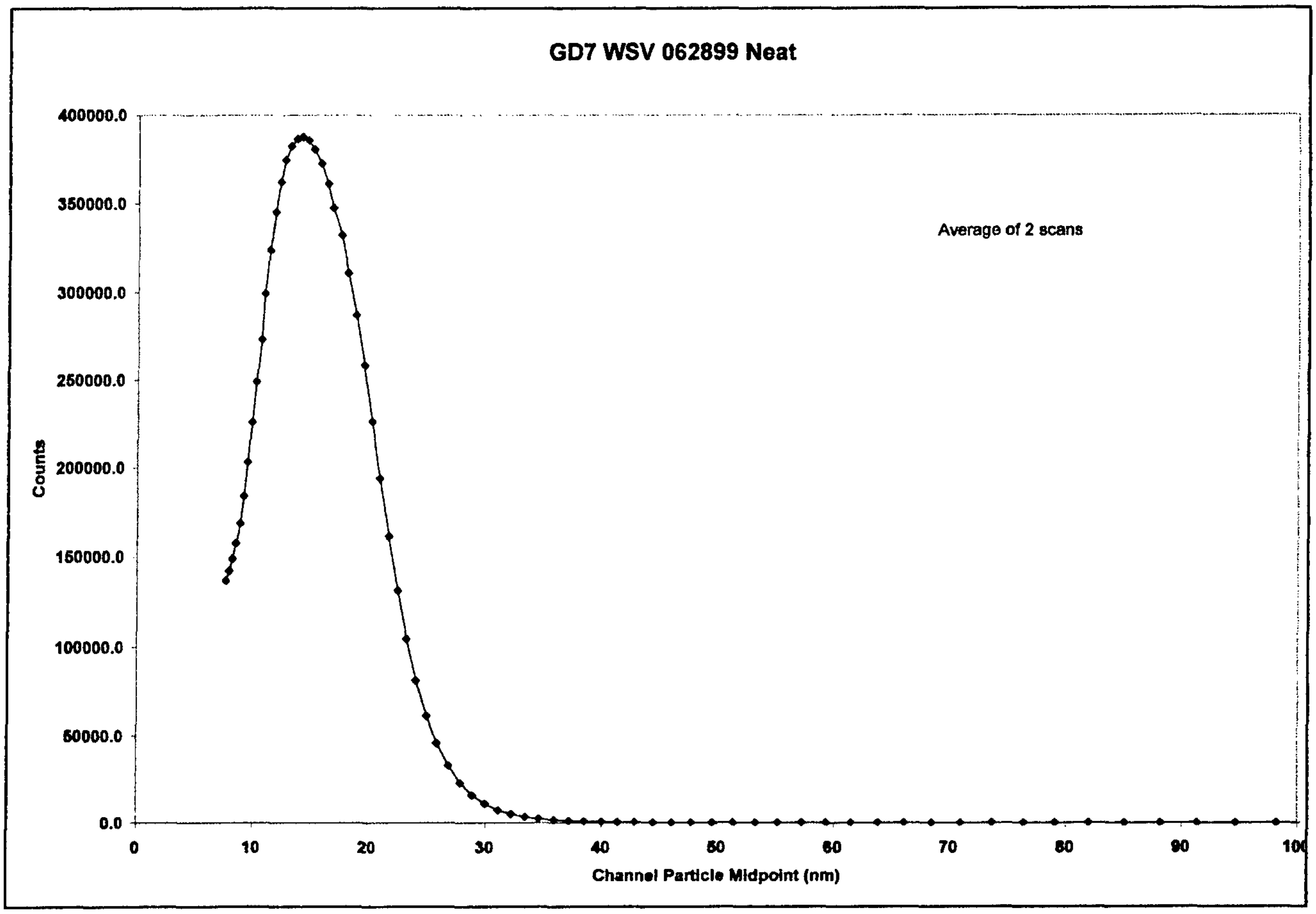

Figure 12: IVDS Output of GD7 Picornavirus As Received from Charles River Laboratory

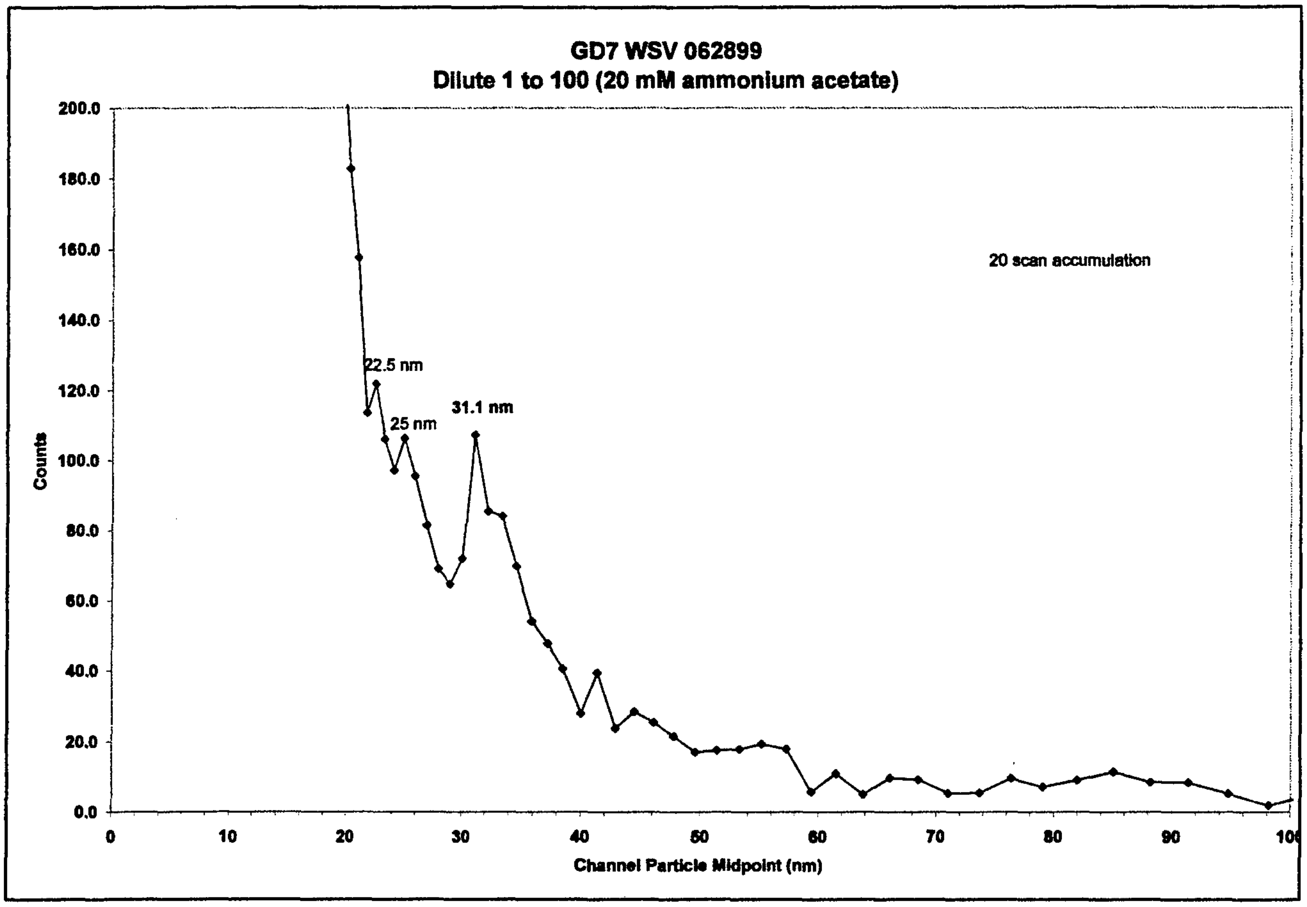

Figure 13: IVDS Output of GD7 Picornavirus after Dilution with 20 mM Ammonium Acetate

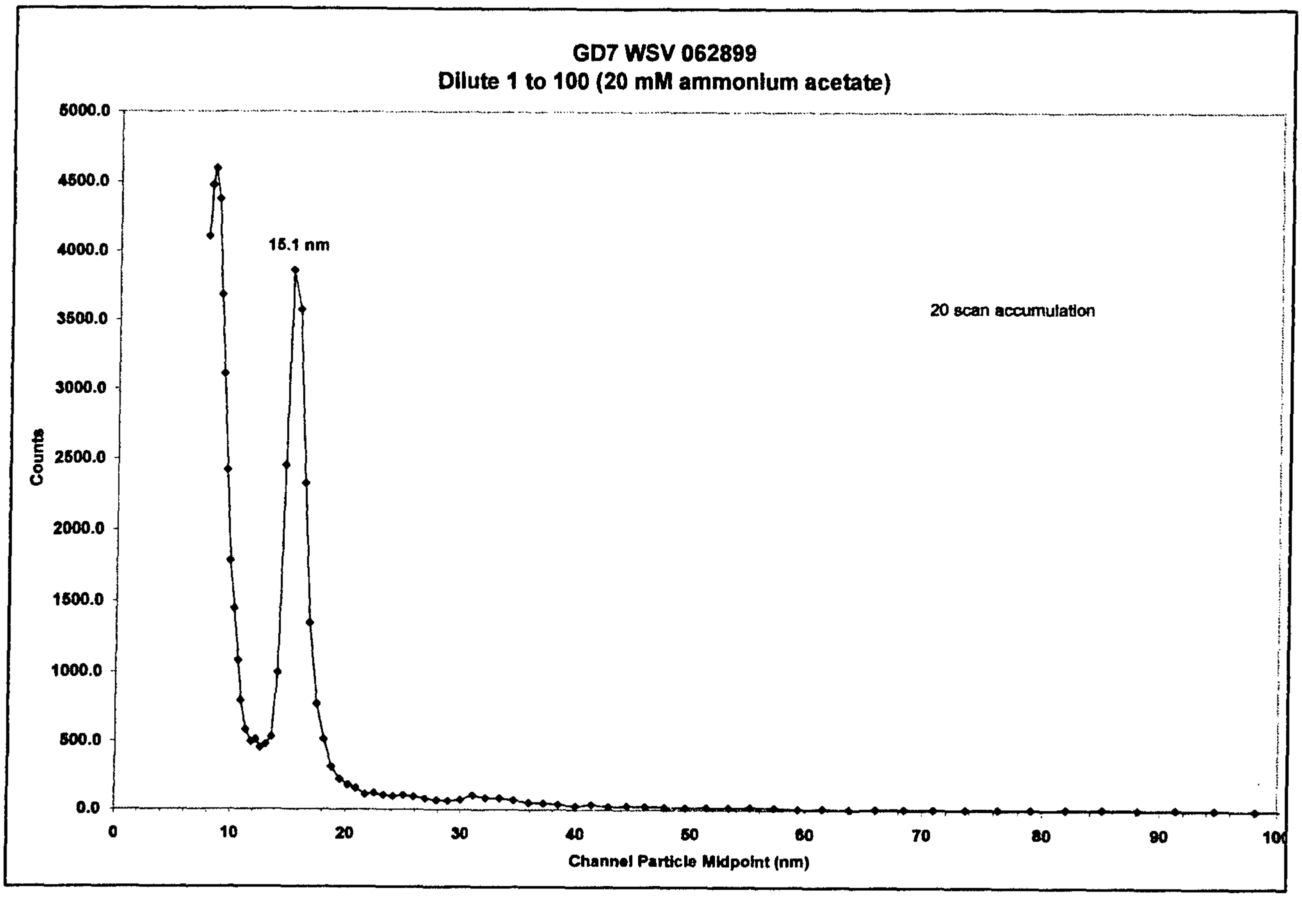

Figure 14: Full-Scale Expansion of Figure 13 (Diluted GD7 Picornavirus Sample)

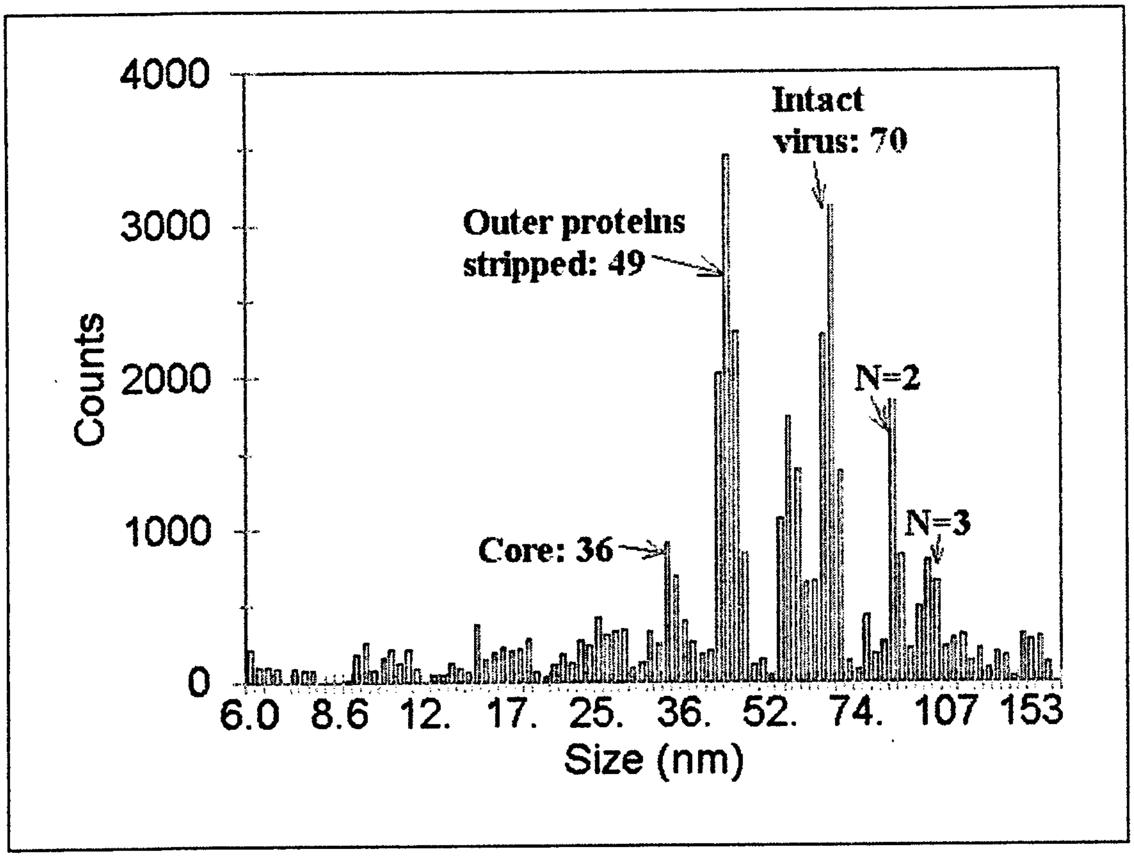

Figure 15: Example of Scan of Alphavirus

UF-GEMMA Analysis of MS2:

Intact virions, RNA, and coat proteins detected

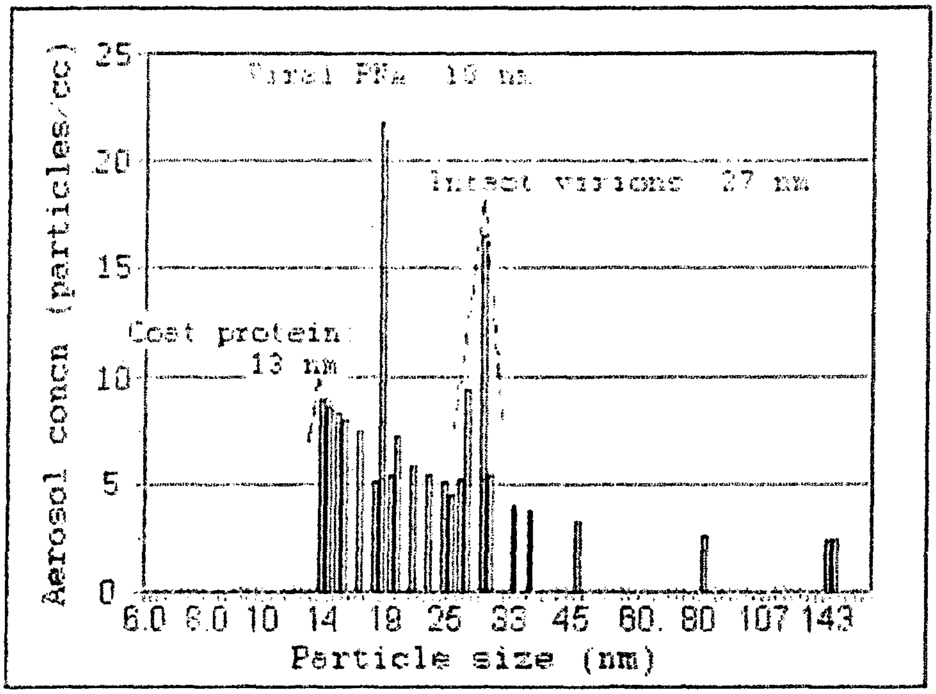

Figure 16: MS-2 and Its Building Blocks as seen by the IVDS

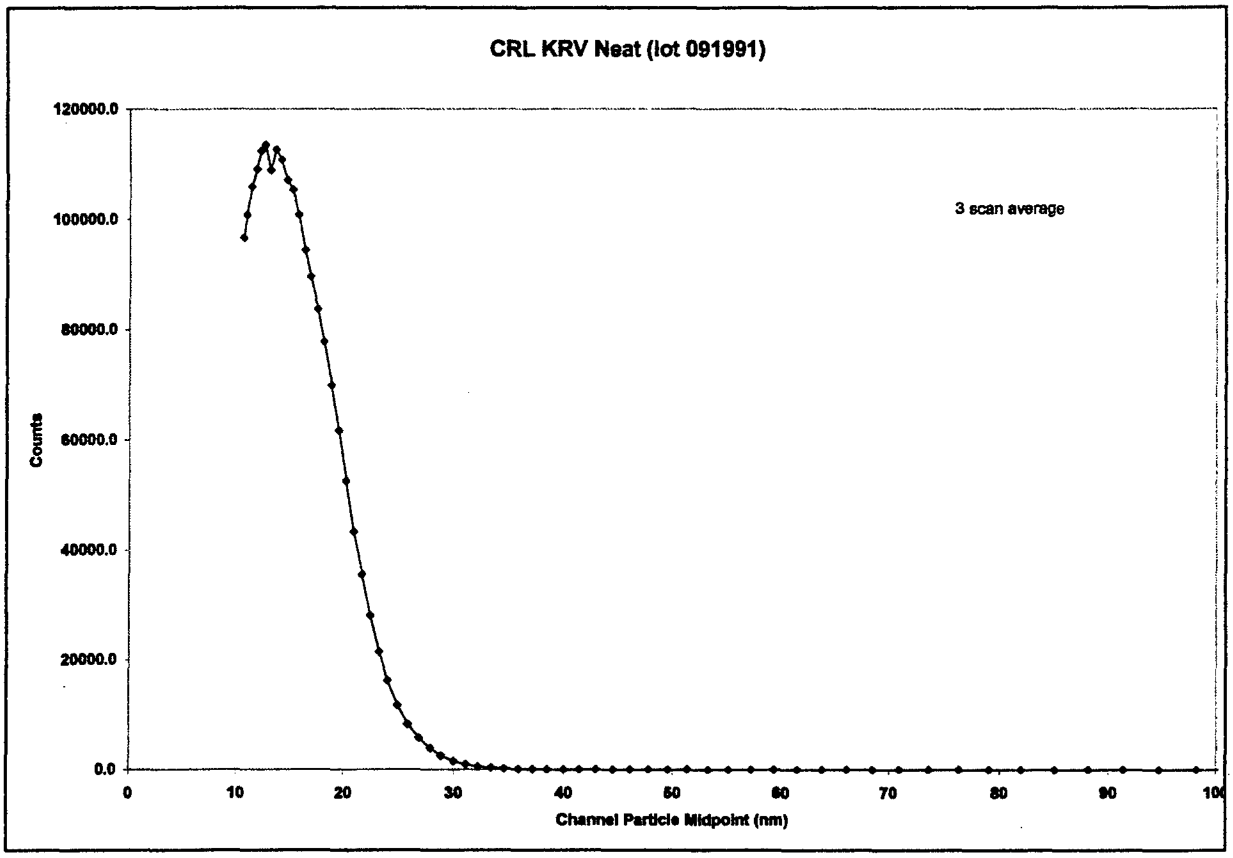

Figure 17: IVDS Run of Neat Kilham Rat Virus Solution

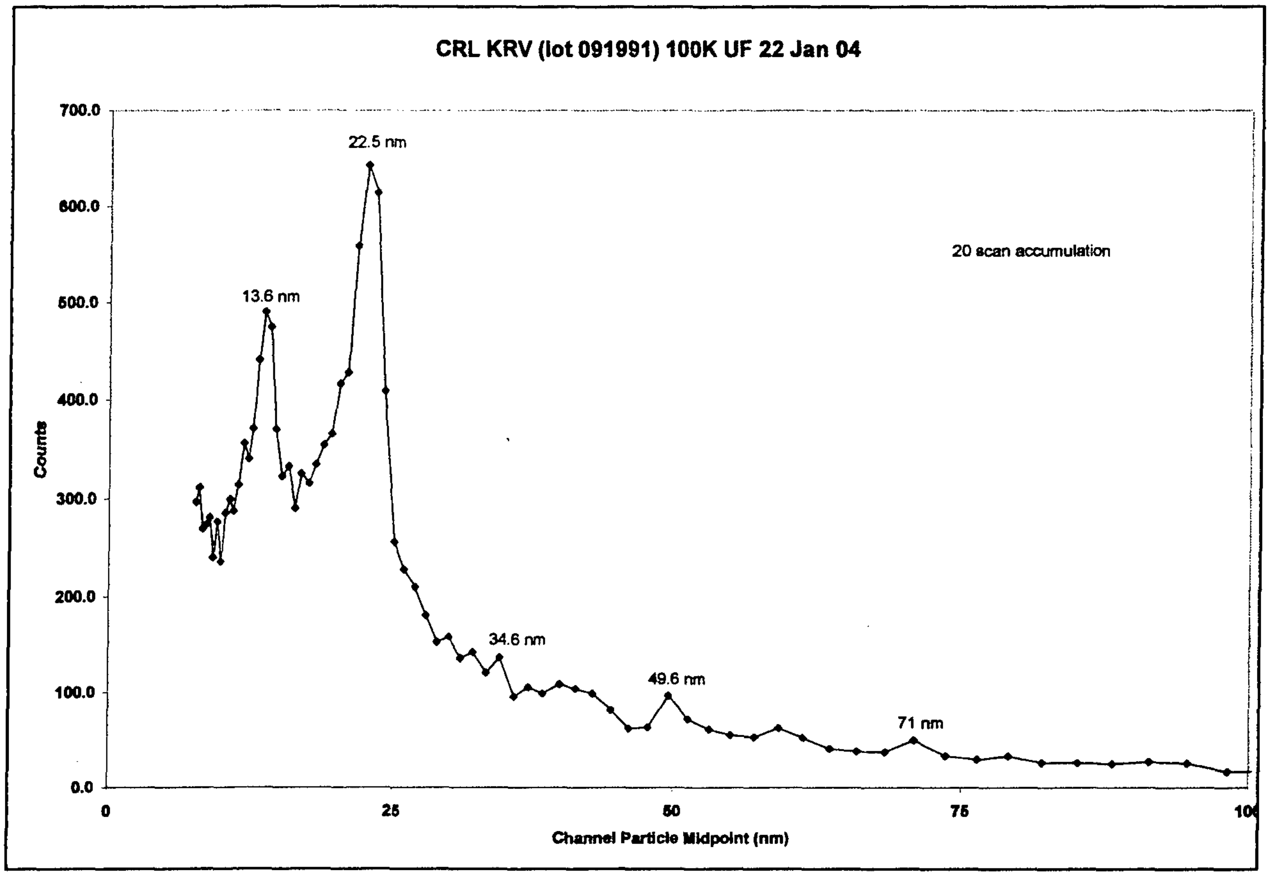

Figure 18: IVDS Run of Diluted Kilham Rat Virus Solution

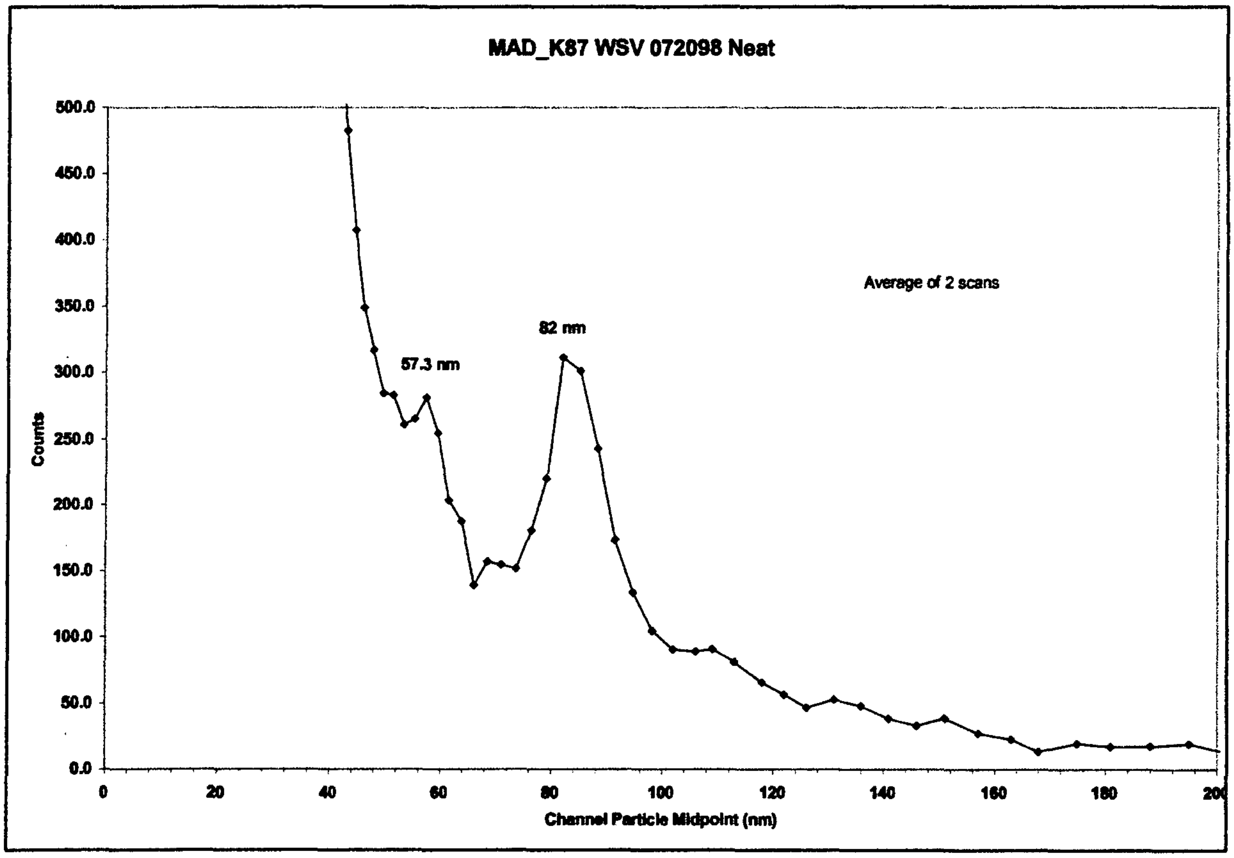

Figure 19: MAD-K87 Virus-Neat, Expanded Scale

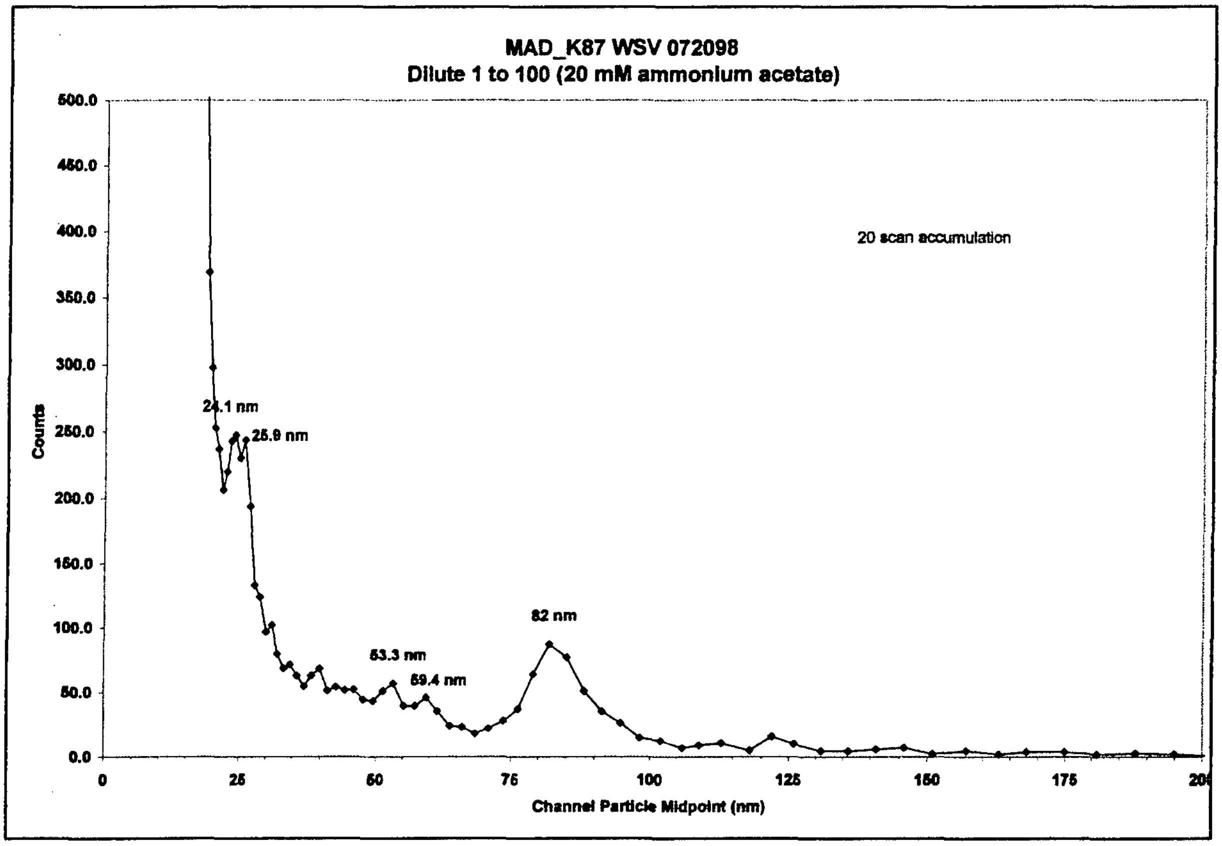

Figure 20: MAD-K87 Virus-Diluted and Concentrated, Expanded Scale

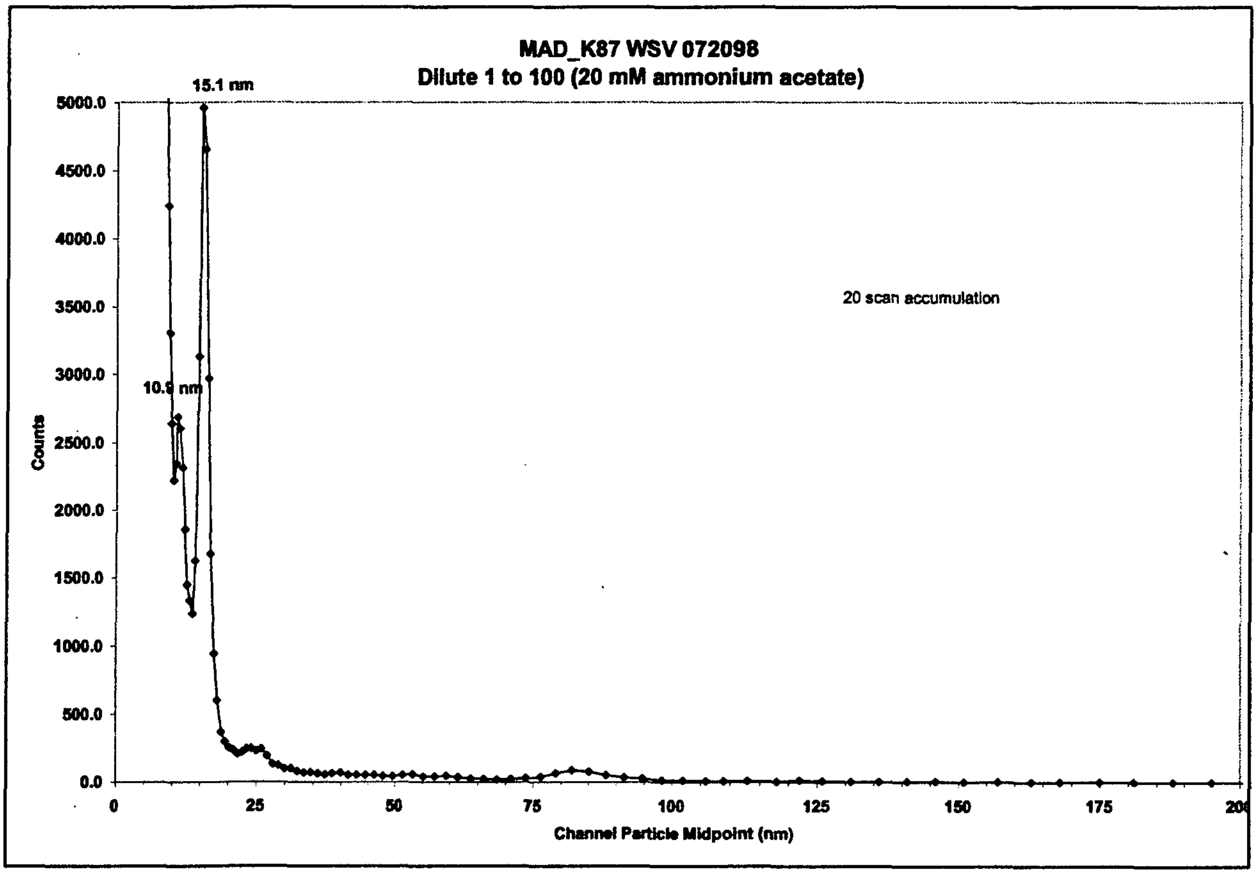

Figure 21: MAD-K87 Virus-Diluted and Concentrated, Expanded Scale

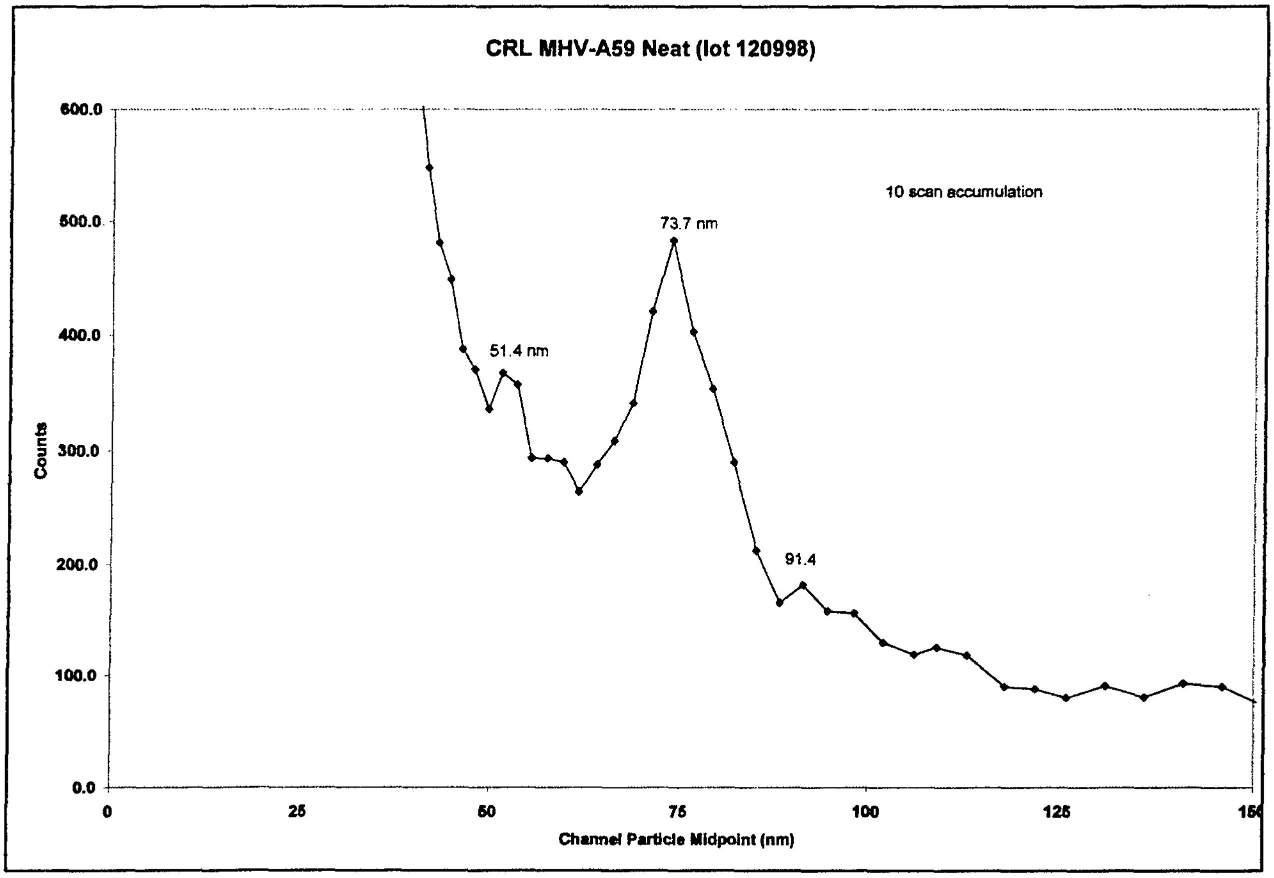

Figure 22: Mouse Hepatitis Virus MHV-A59 Neat

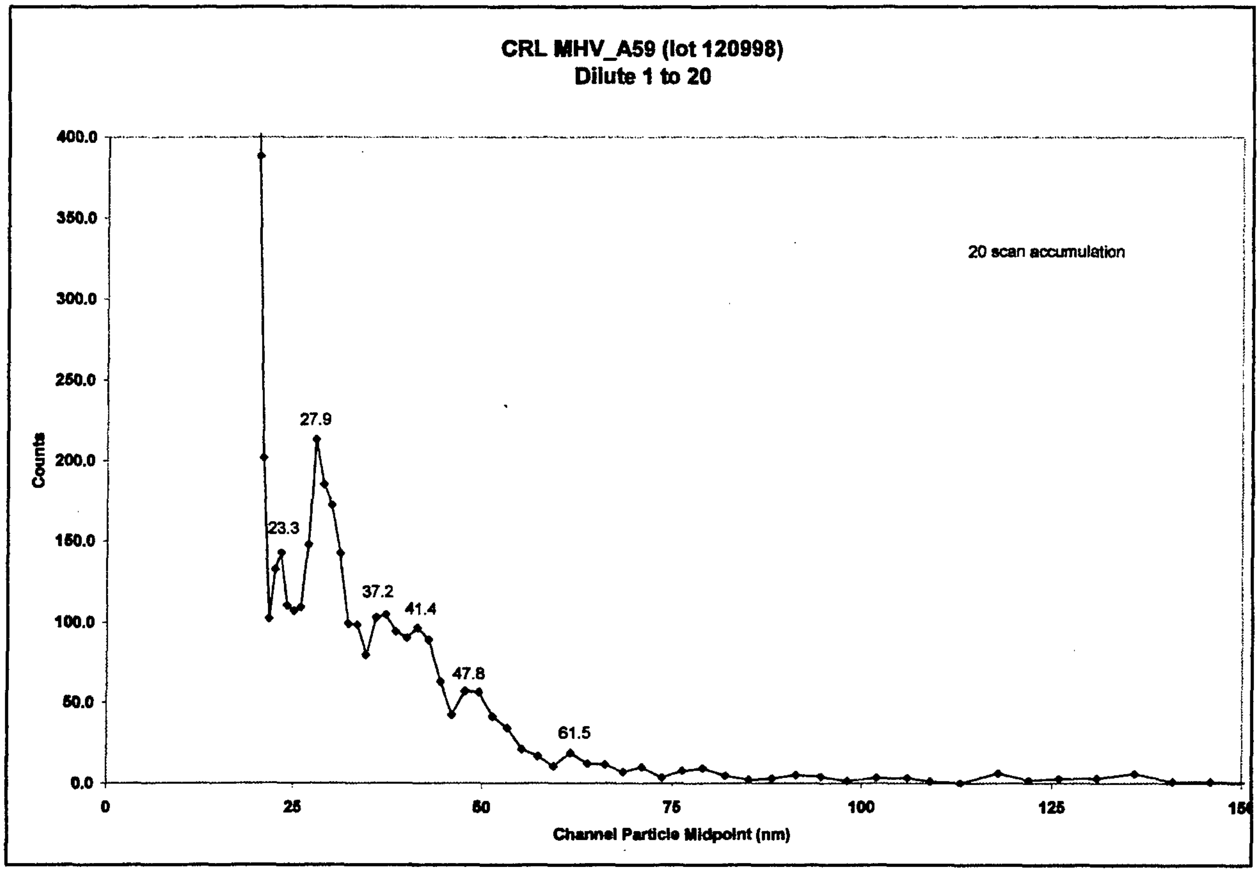

Figure 23: Mouse Hepatitis Virus MHV-A59 Diluted with Ammonium Acetate

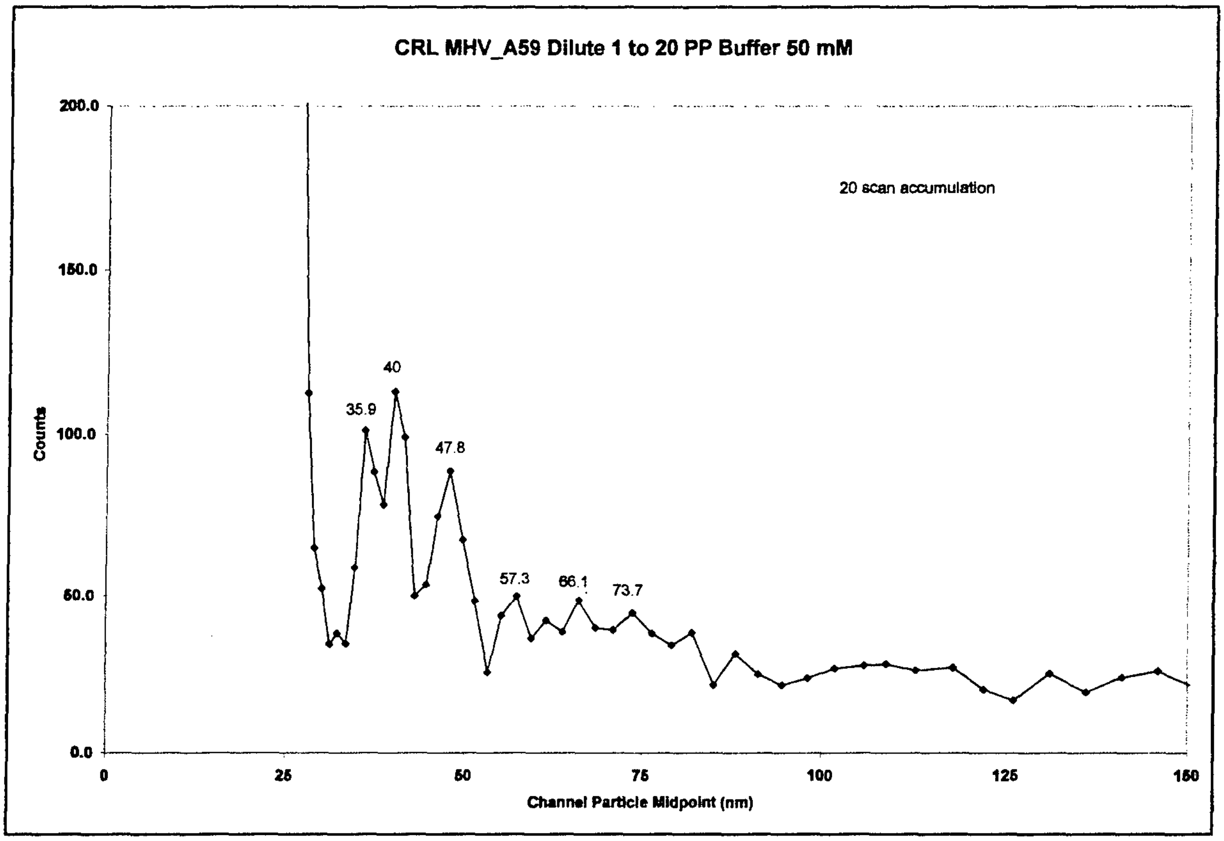

Figure 24: Mouse Hepatitis Virus MHV-A59 Diluted with Potassium Phosphate

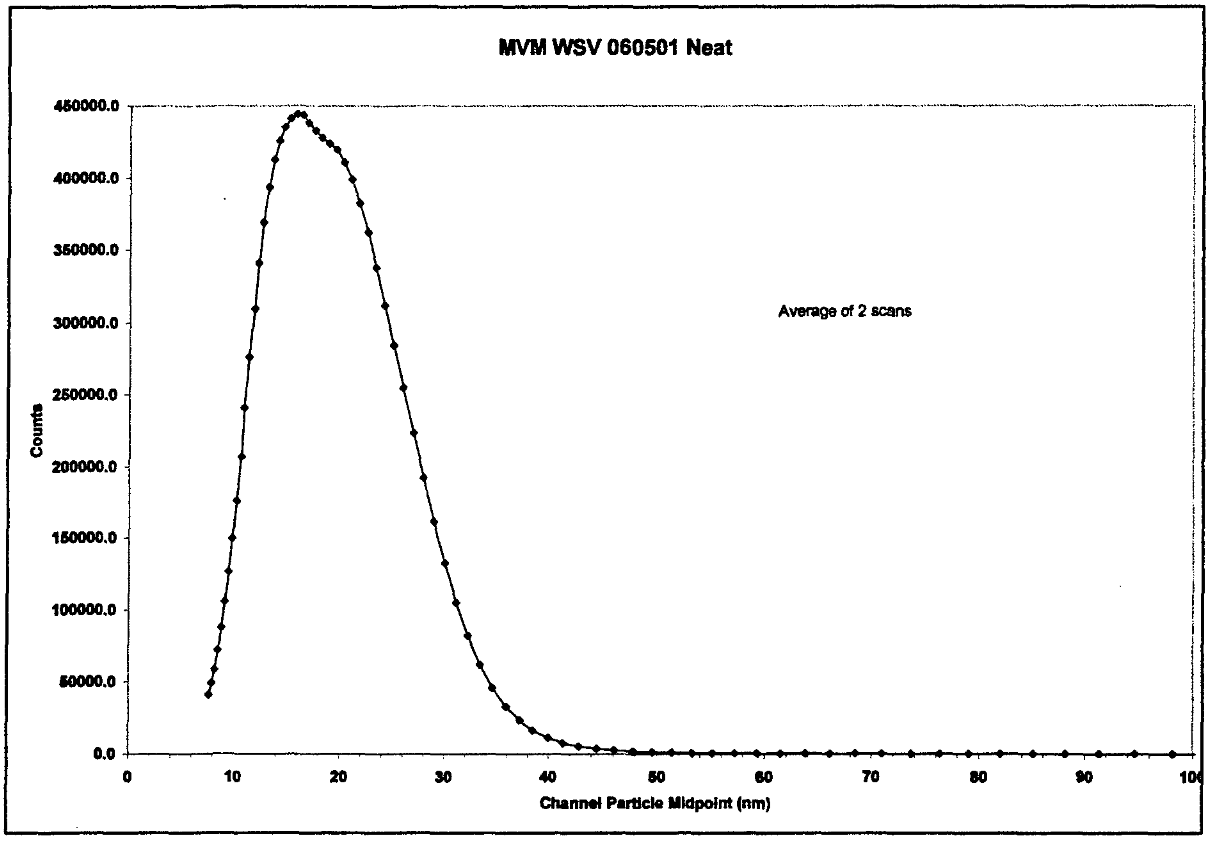

Figure 25: Scan of MVM Parvovirus - Neat

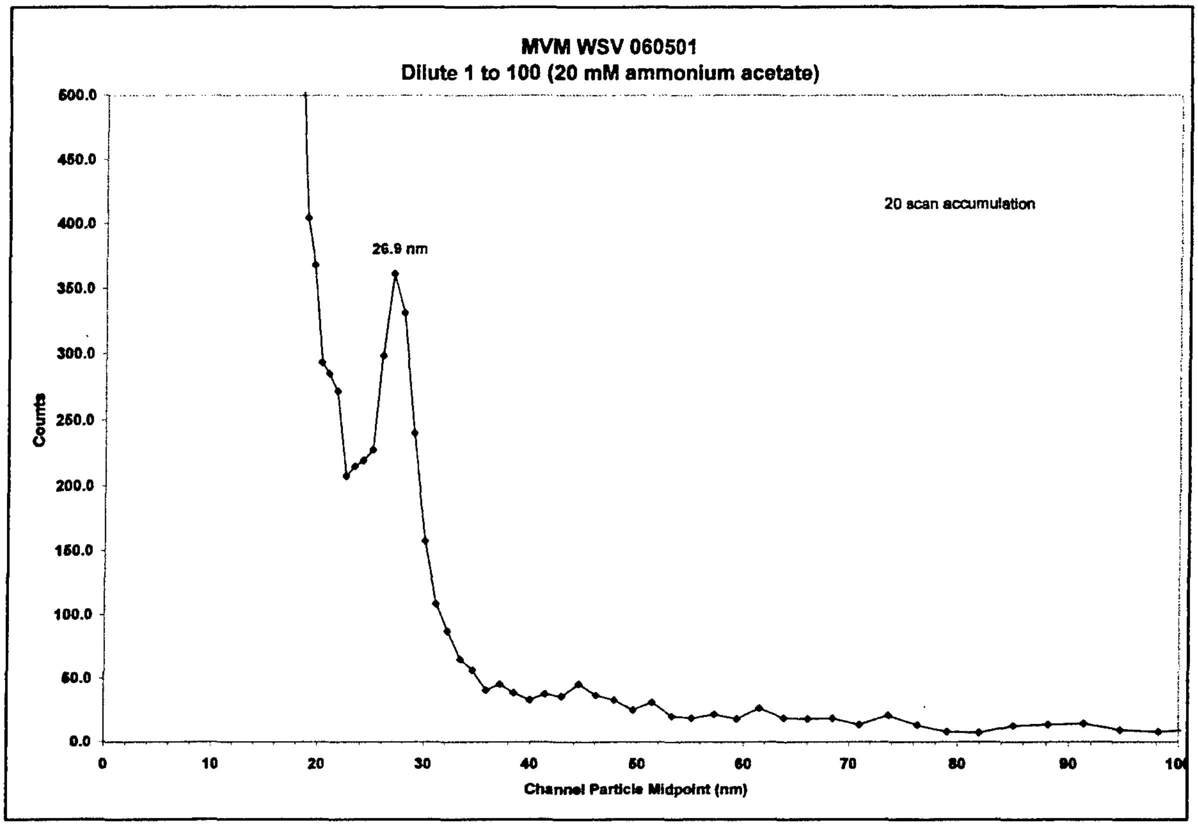

Figure 26: Scan of MVM Parvovirus-Diluted in Ammonium Acetate, Expanded Scale

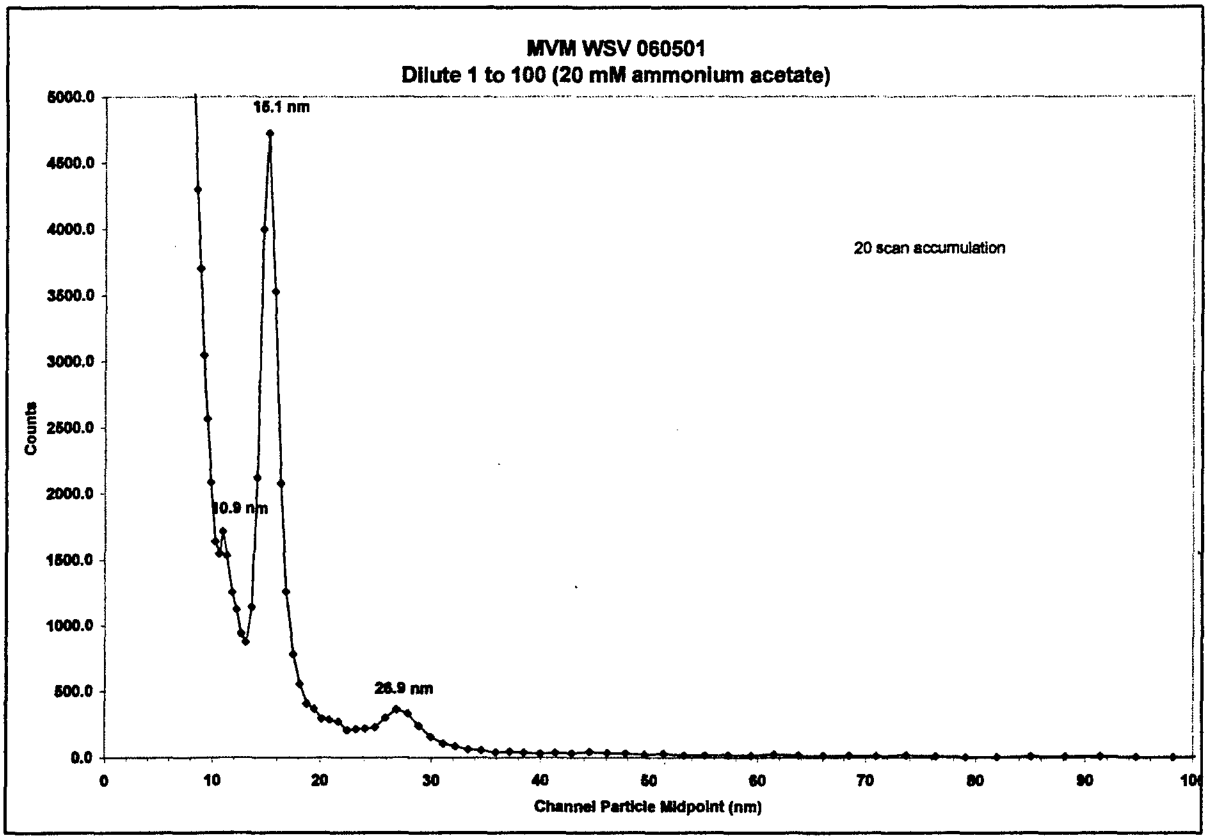

Figure 27: Scan of MVM Parvovirus-Diluted in Ammonium Acetate, Full Scale

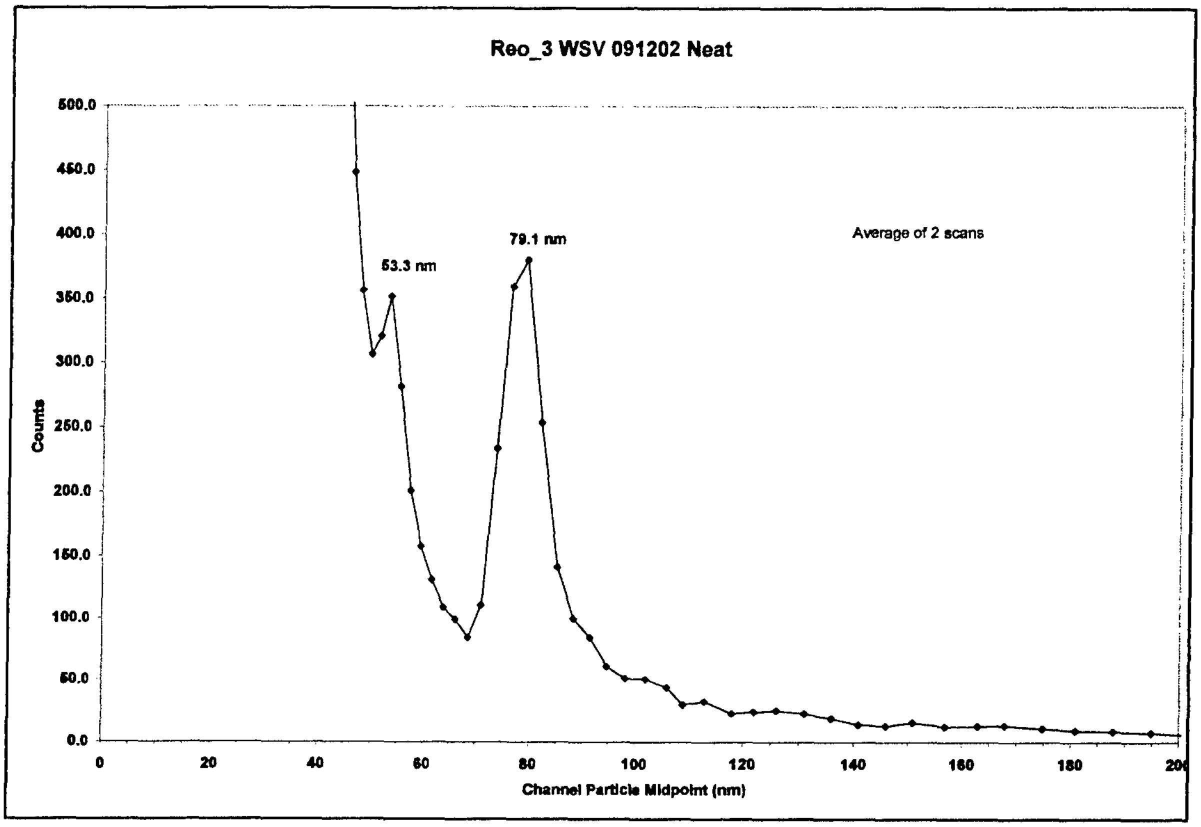

Figure 28: Scan of Reo-3 Reovirus - Neat

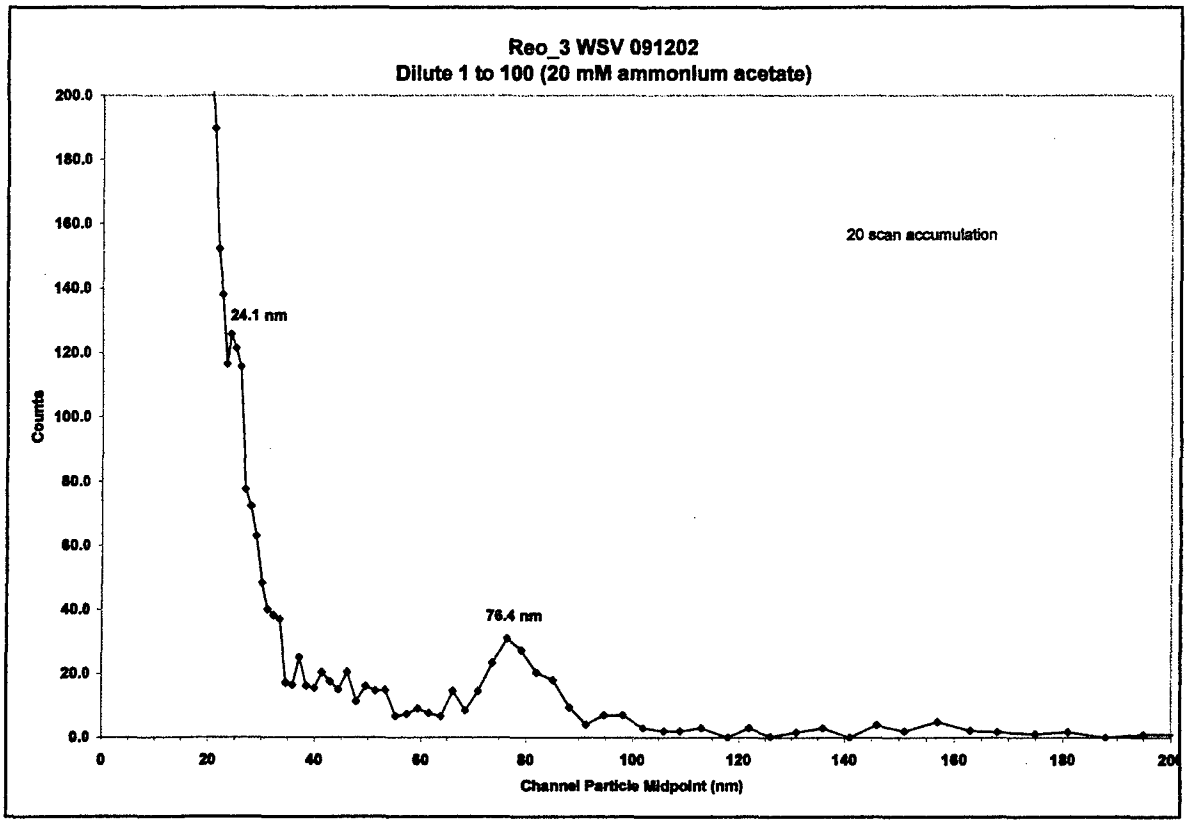

Figure 29: Scan of Reo-3 Reovirus Diluted in Ammonium Acetate

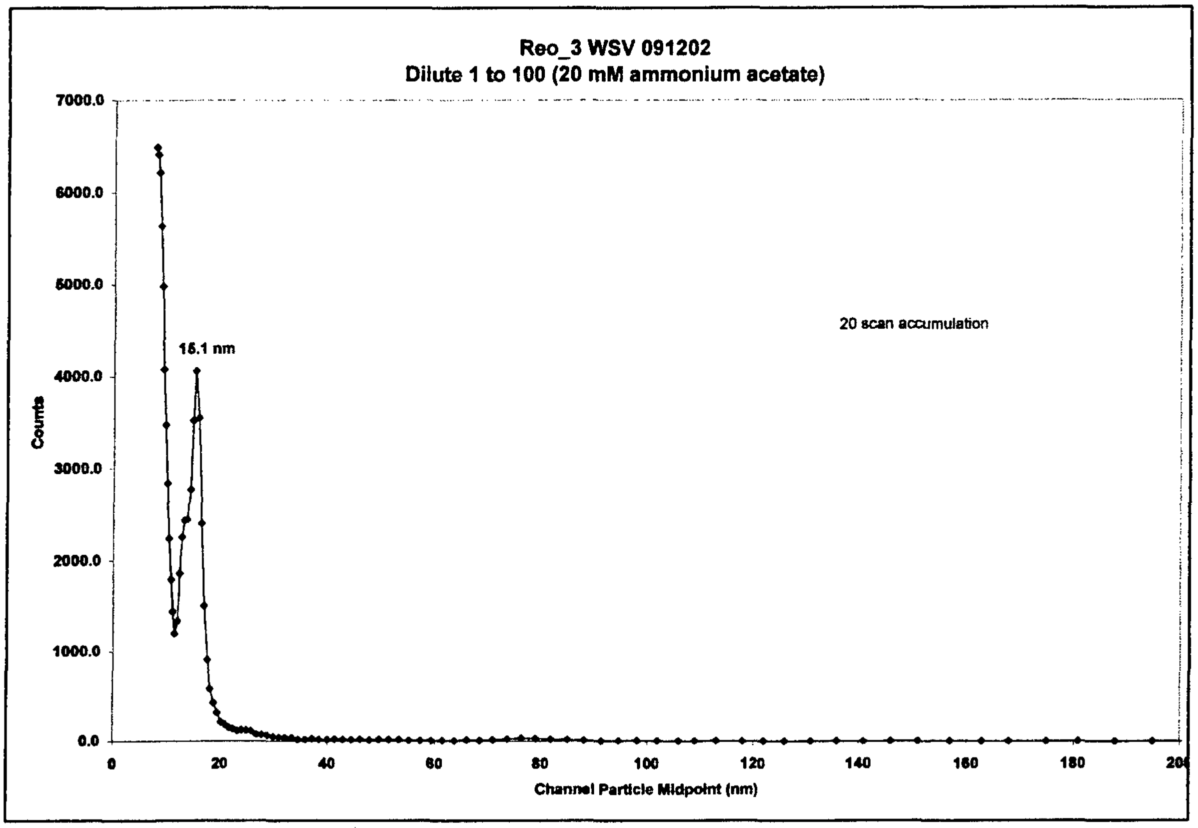

Figure 30: Scan of Reo-3 Reovirus Diluted in Ammonium Acetate, Full Scale

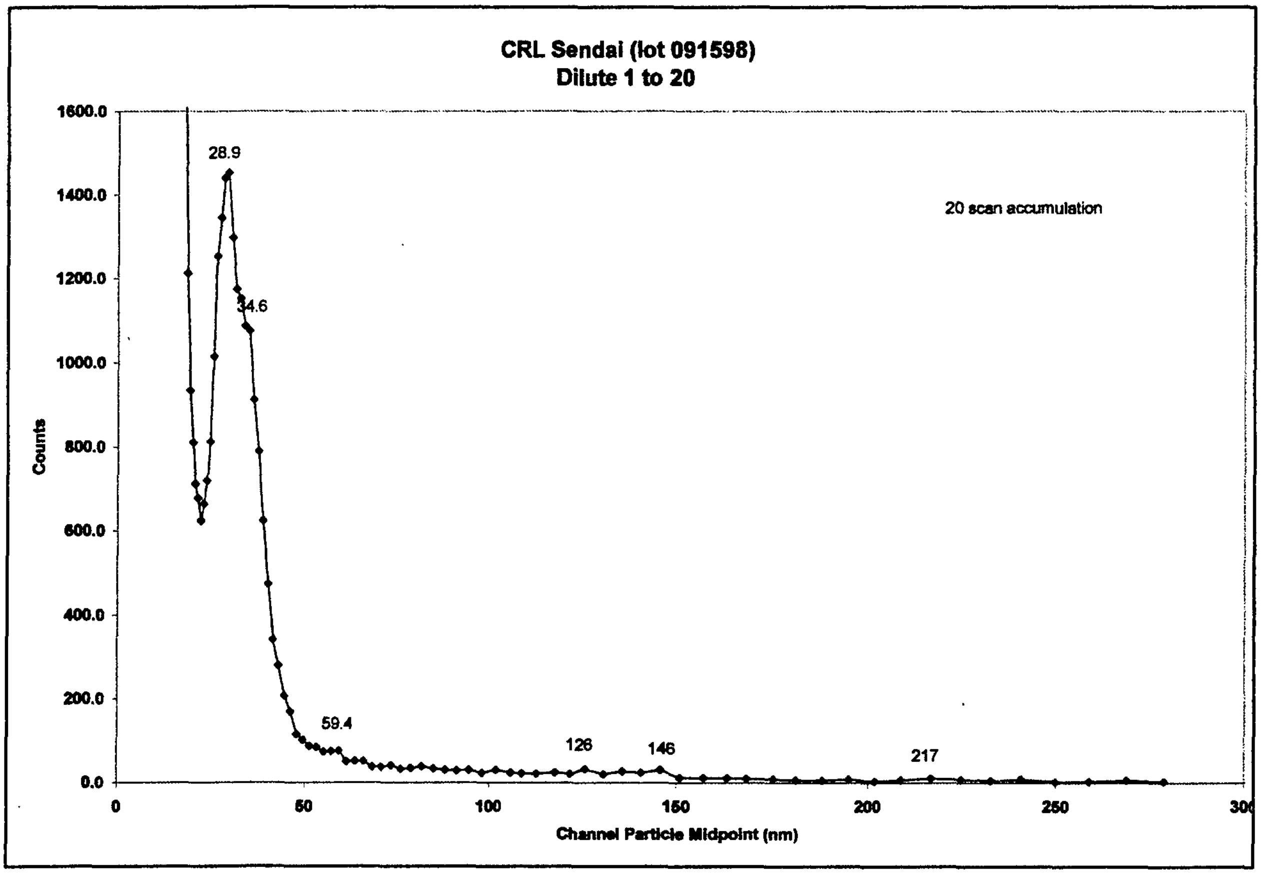

Figure 31: Sendai Rodent Virus Diluted with Ammonium Acetate

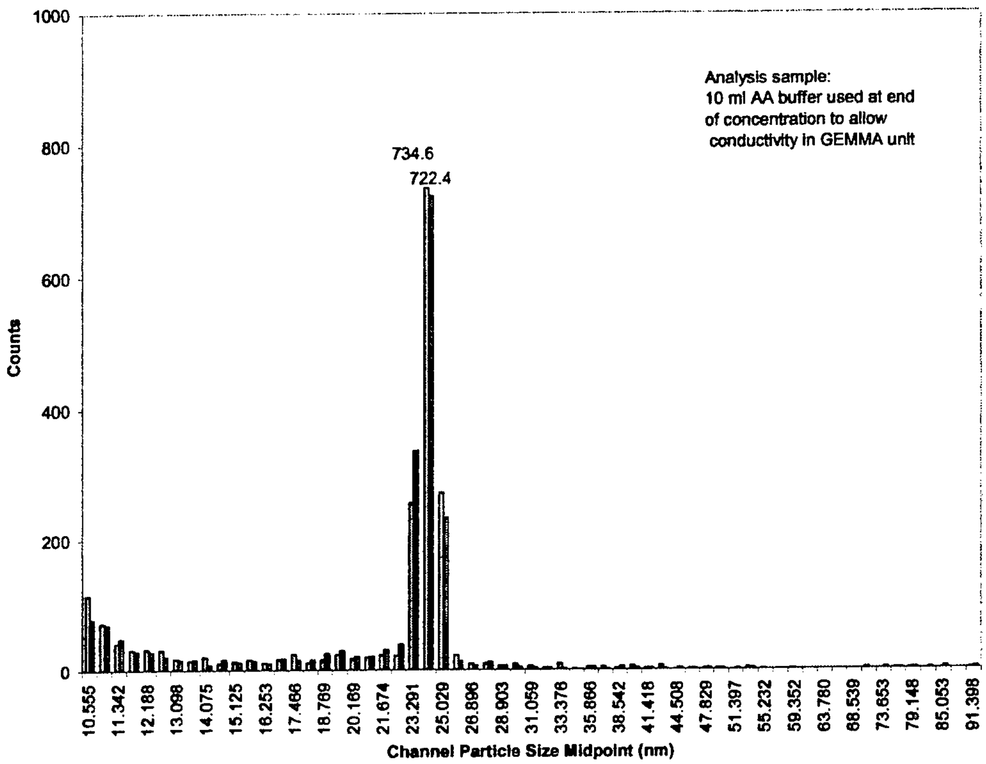

Figure 32: Analysis of MS-2 in Water.

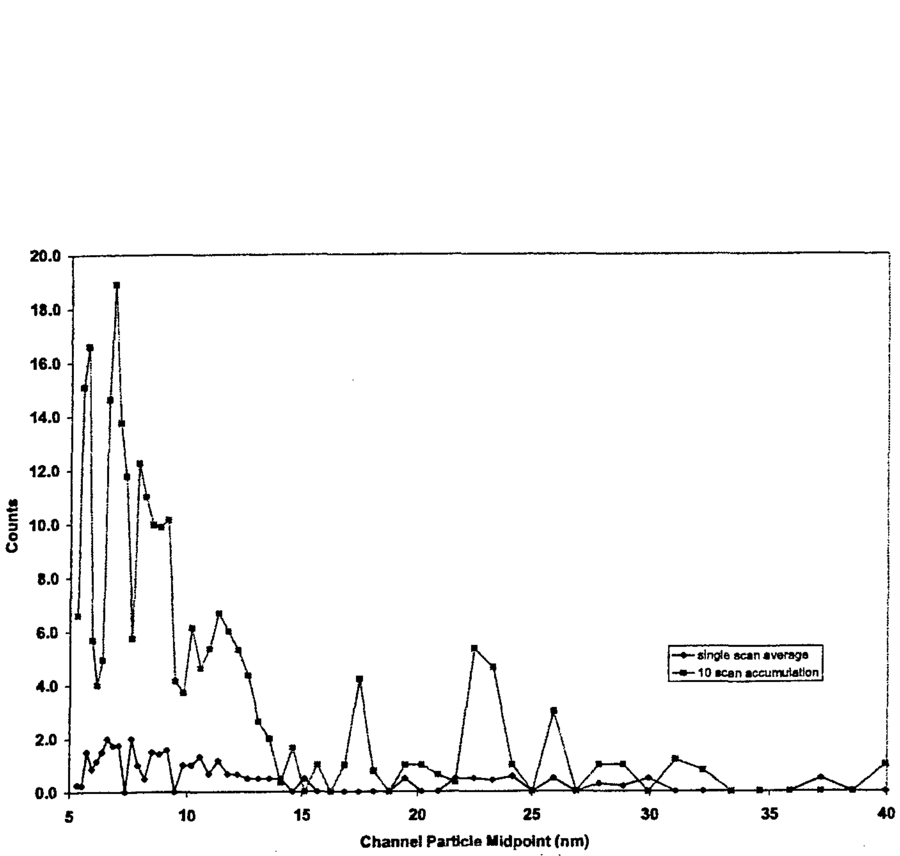

Figure 33: Analysis of MS-2 Mixed in Water As Is

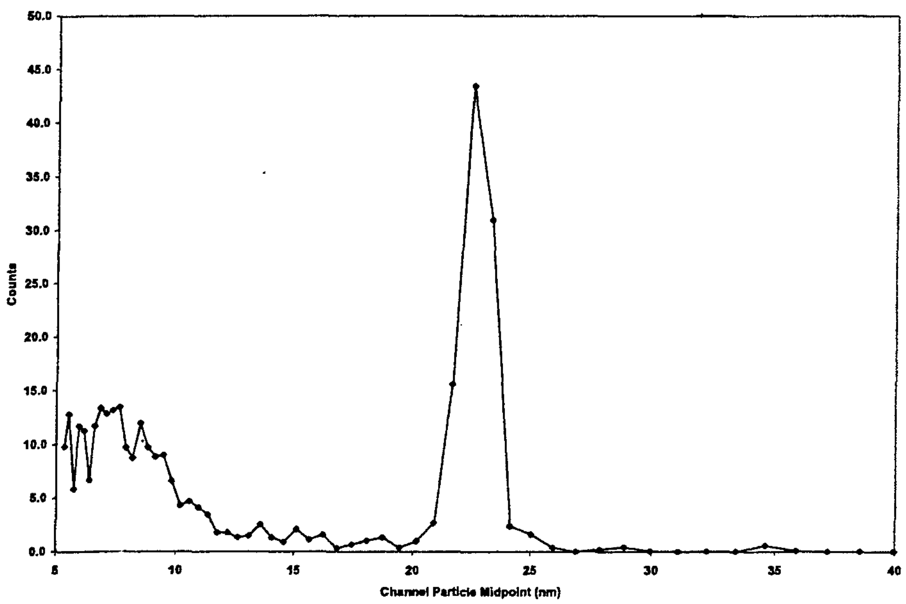

Figure 34: Analysis of MS-2 Mixed in Water after Filtration and Concentration

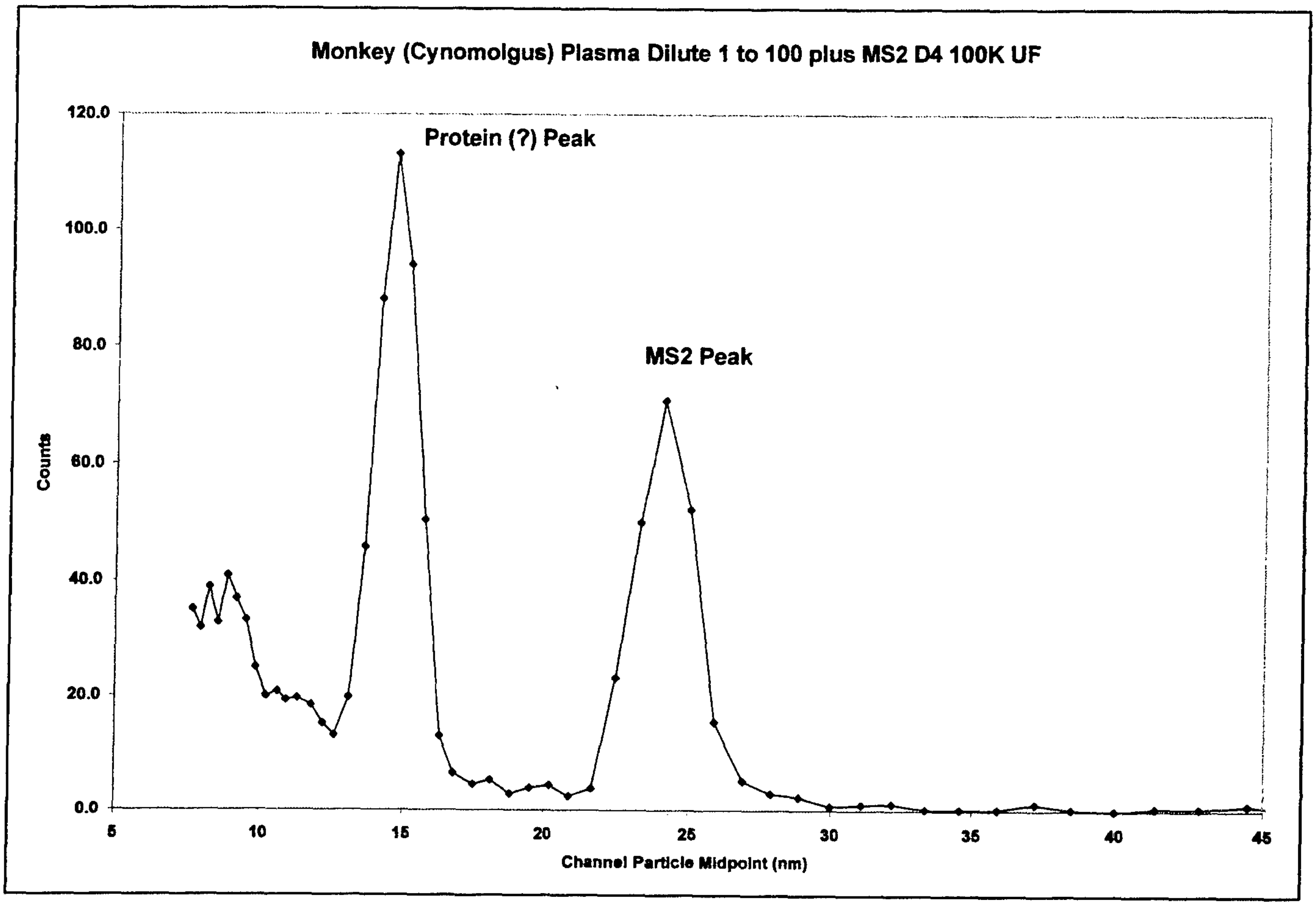

Figure 35: Recovery of MS-2 from Spiked Monkey Plasma

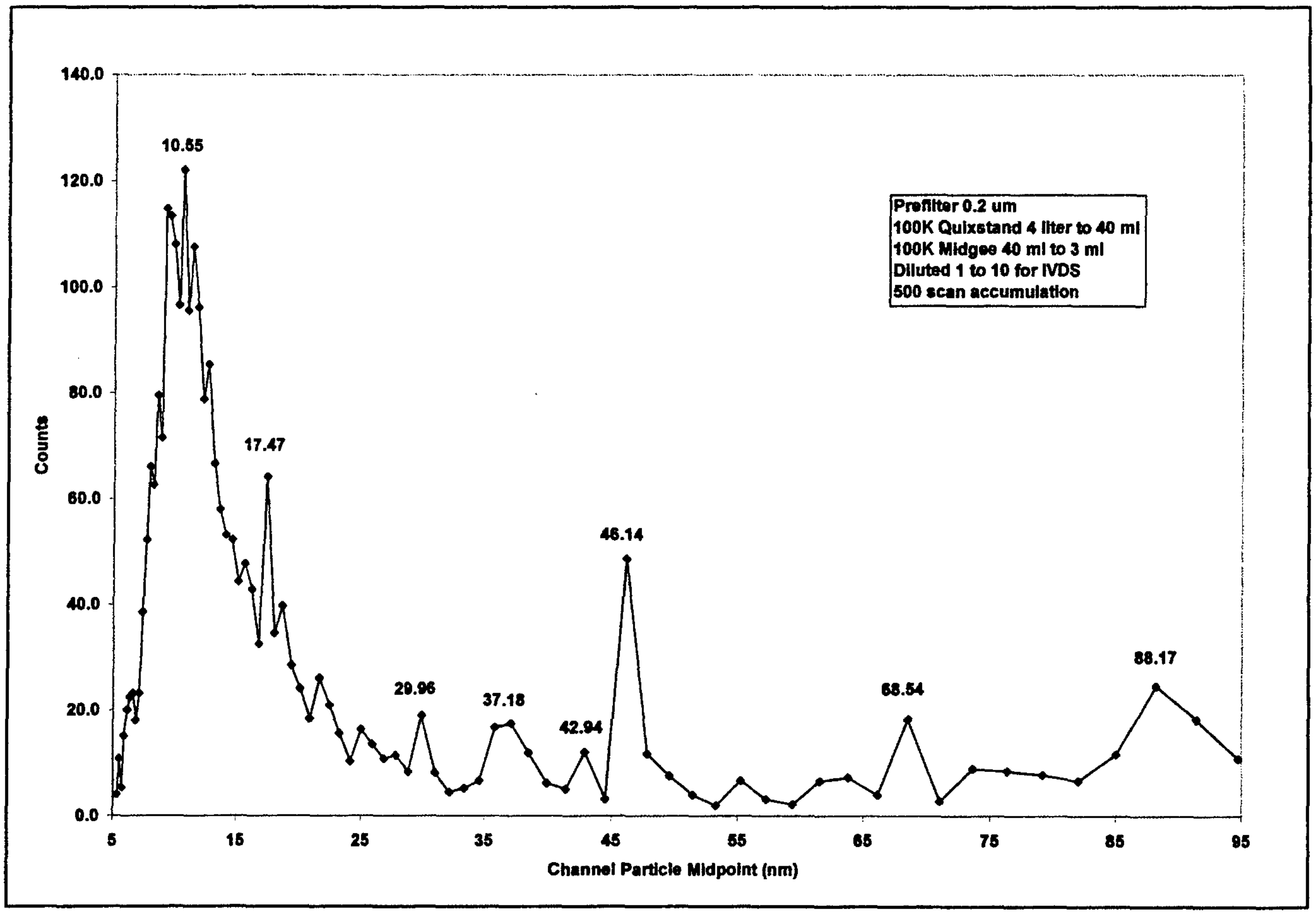

Figure 36: Results of Analysis of Sea Water by the IVDS

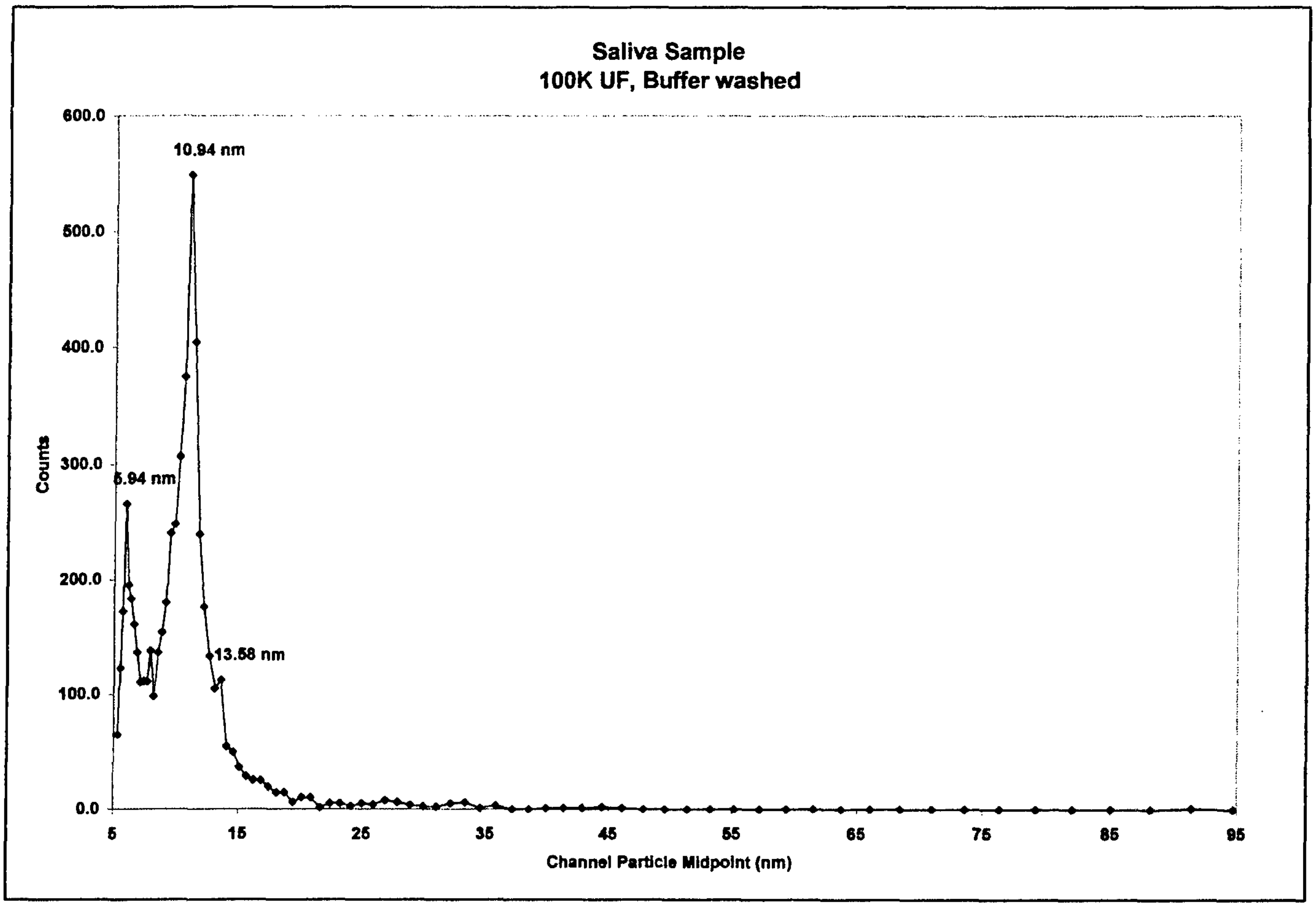

Figure 37: Scan of Saliva Sample

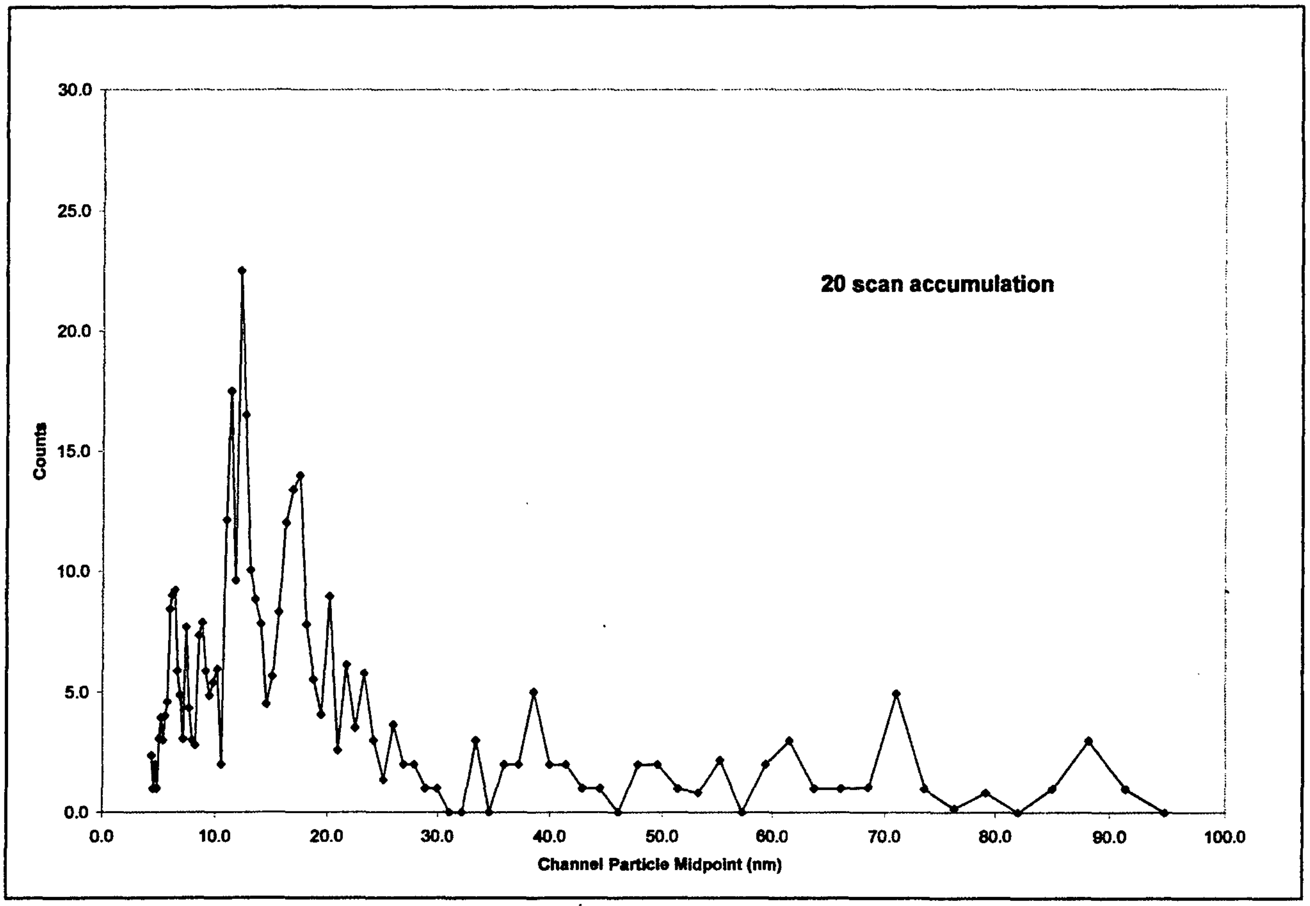

Figure 38: IVDS Scan of an Environmental Air Sample

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LITERATURE CITED

1. *Optimisation of a New Method for Detection of Viruses in Groundwater*; National Groundwater and Contaminated Land Centre, July 2000.

2. http://www8.medica.de/cipp/md_medica/custome/pub/content,lang,2/ticket,g_a_s_t/oid,11978/parent,5965/local_lang,2. Accessed 22 November 2004.

3. Wick, C.H.; Anderson, D.M.; McCubbin, P.E. *Characterization of the Integrated Virus Detection System (IVDS) using MS-2 Bacteriofage*; ECBC-TR-018; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1999; UNCLASSIFIED Report (AD-A364 117).

4. Wick, C.H.; Anderson, D. *System and Method for Detection, Identification, and Monitoring of Sub-Micron Sized Particles*. U.S. Patent 6,051,189, others pending.

5. Wick, C.H.; Elashvili, I.; McCubbin, P.E. *Analysis of The Physical Behavior Of Viruses Using The Integrated Virus Detection System (IVDS)*. Paper presented at The 23rd Army Science Conference; Orlando, FL, 2002.

6. Fuchs, N.A. *The Mechanics of Aerosols*; Pergamon: New York, 1964.

7. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/52030001.htm accessed December 2004.

8. http://www.ncbi.nlm.nih.gov.ICTVdb/ICTVdB/00.050.1.htm accessed December 2004.

9. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/01010000.htm accessed December 2004.

10. http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_coron.htm accessed December 2004.

11. Birenzvige, A., Wick, C.H., *The Truth about Bioterrorism; In Forensic Aspects of Chemical and Biological Terrorism*; Wecht, C.H., Ed.; Lawyers & Judges Publishing Company, Inc.: Tuscon, AZ, 2004.

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APPENDIX

KNOWN PROPERTIES OF VIRUSES ANALYZED BY THE IVDS

A-1. GD7 PICORNAVIRUS-ICOSAHEDRAL VIRUS

Theiler's Meningoenchephalomyelitas Virus Gr.

Picornavirus-icosahedral, 22-30 nm

Cardiovirus

Taxonomy

Virus Code. 52.0.4. *Virus Accession number* 52040000.
Synonym(s): EMC virus group.

Comments: Mengovirus, Columbia SK virus and mouse Elberfield virus are best regarded as strains of EMCV, based on serological cross-reaction and sequence identity. Theiler's encephalomyelitis virus, also known as murine poliovirus, lacks a poly (C) tract but has 54% nucleotide sequence identity with EMCV and < 40% with other picornavirus groups. The location and nature of its antigenic sites are comparable to those of the other cardioviruses.

Virus infects vertebrates.

Description is on taxonomic level of genus. Virus belongs to the family *Picornaviridae* (VC 52.).

Properties of Virion

Morphology

Virions not enveloped. Nucleocapsids isometric; 30 nm in diameter. Symmetry icosahedral. Nucleocapsids appear to be round. 12 capsomers per nucleocapsid.

Nucleic Acid

Virions contain 33 % nucleic acid. Virions contain one molecule of linear positive-sense single stranded RNA.

Total genome length is 8400 nt. The 5' end of the genome has a genome-linked protein (VPg). 3' end has a poly (A) tract.

Lipids

Virions contain 0 % lipid.

Genome Organization and Replication

Genomic nucleic acid infectious.

Taxonomic Structure

Taxonomic structure of the genus.
Type species: 52.0.3.0.001 encephalomyocarditis virus, *syn.* Columbia SK virus; mengovirus; mouse Elberfield virus.
Species: 52.0.3.0.002 Theiler's murine encephalomyelitis virus, *syn.* murine poliovirus.

Reference

The following reference is cited in the *Sixth ICTV Report*: rf_picor.htm.

Data sources and contributors.

The above description has been compiled from the data presented in the *Sixth ICTV Report* by Minor PD, Brown F, Domingo E, Hoey E, King A, Knowles N, Lemon S, Palmenberg A, Rueckert RR, Stanway G, Wimmer E, Yin-Murphy M.

A-2. PARVOVIRUS

Cite this publication as: Büchen-Osmond, C. (Ed), (2003). 00.050.1.01. *Parvovirus*. In: *ICTVdB - The Universal Virus Database*, version 3. Büchen-Osmond, C. (Ed), ICTVdB Management, The Earth Institute, Biosphere 2 Center, Columbia University, Oracle, AZ, USA

Cite this site as: *ICTVdB - The Universal Virus Database*, version 3. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/

Classification

This is a description of a vertebrate virus at the genus level.

ICTVdB *Virus Code*: 00.050.1.01. *Virus accession number*: 050101GE. Former *Virus Code*: 50.1.1.; *former accession number*: 50110000. TaxID: [10781].

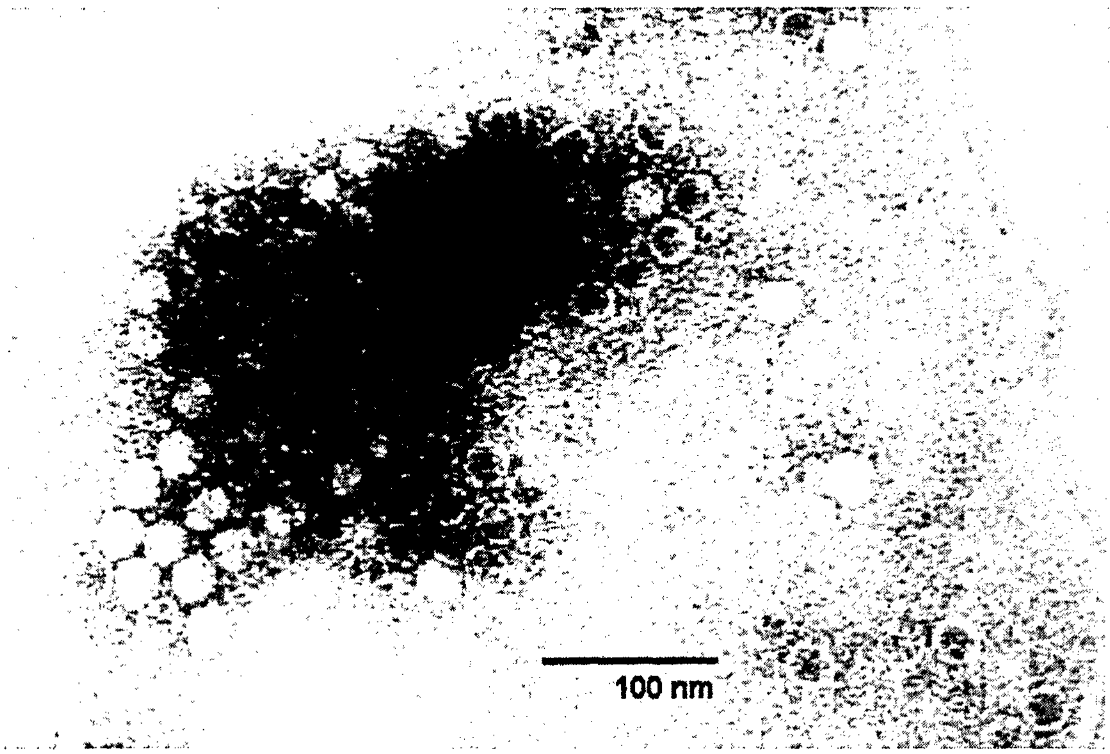
Name, Synonyms and Lineage

Virus is assigned to the subfamily 00.050.1. *Parvovirinae*; assigned to the family 00.050. *Parvoviridae*.

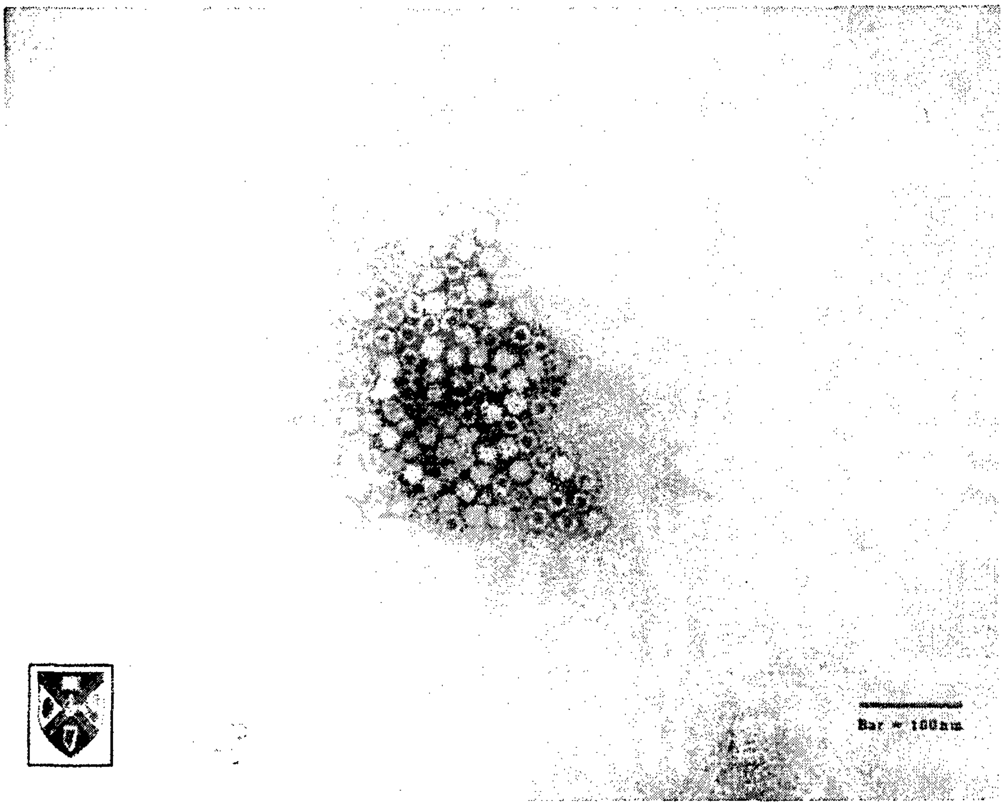
Morphology

Virions have a simple construction and consist of a capsid. Virions are not enveloped. Capsid is round and exhibits icosahedral symmetry. The nucleocapsid is isometric and has a diameter of 20–26 nm. Capsids appear round. The capsid consists of 60 capsomers (each a quadrilateral 'kite-shaped' wedge). Surface projections are small and surface appears rough and distinct spikes.

Capsids can be penetrated by stain, some appear dark in the center and only one species is recovered in preparations, or virus may occur together with a dependent virus.



Canine parvovirus. Electron Micrograph by C Büchen-Osmond. For enquiries contact C Büchen-Osmond.



Parvovirus: Electron Micrographs of Animal Viruses, Veterinary Sciences, The Queen's University of Belfast, kindly provided by Prof Stewart McNulty

3D image of canine parvovirus. Reconstruction by Jean Yves Sgro, Institute of Molecular Virology, University of Madison.

Nucleic Acid

The genome is not segmented and consists of a single molecule of linear negative-sense, or negative-sense and positive-sense (up to 50% in some members) single-stranded DNA. The complete genome is 5000 nucleotides long. The genome has terminally redundant sequences. The genome sequence is repeated at both ends. Nucleotide sequences at the 3'–terminus are complementary to similar regions on the 5' end, or unrelated to the 5'–terminus. The 5'–terminal sequence has palindromic repeats, forming a hairpin structure.[2] Terminal repeats at the 5'–end are 200–242 nucleotides long. The 3'–terminus has conserved nucleotide sequences that are 115–116 nucleotides in length in species of same genus. Populations of mature viruses contain particles with equivalent numbers of positive and negative sense ssDNA . Upon extraction, the complementary DNA strands usually

form dsDNA.
Reference(s) to nucleotide sequence: nucleotides at GenBank; complete genomes.

Proteins

The viral genome encodes structural proteins.

Lipids

Lipids are absent.

Translation: Virions are associated with helper virus, but independent from its functions during replication.

Biological Properties

Natural Host Range

Virus infects during its life cycle a single type of vertebrate host. Viral hosts belong to the Domain Eucarya.

Domain Eucarya
Kingdom Animalia.

Kingdom Animalia
Phylum Chordata.

Phylum Chordata
Subphylum Vertebrata; Class Mammalia.

Class Mammalia
Order Carnivora, or Rodentia.

Ecology, Epidemiology and Control

A fact sheet on this virus is available from the Long Beach Animal Hospital [http:]]www.lbah.com/Canine/Parvo.html].

Taxonomic Structure of the Genus

Type species 00.050.1.01.001. *Murine minute virus.*

Species in the Genus

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, or subspecies are not italicized.

Virus codes, virus names, arthropod vector and host names { }, serotypes, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.1.01.004.	*Canine minute virus*		(CMV)
00.050.1.01.005.	*Canine parvovirus*	[M19296]	(CPV)
00.050.1.01.006.	*Chicken parvovirus*		(ChPV)
00.050.1.01.007.	*Feline panleukopenia virus*	[M75728]	(FPLV)
00.050.1.01.008.	*Feline parvovirus*		(FPV)
00.050.1.01.010.	*HB virus*		(HBPV)
00.050.1.01.011.	*H-1 virus*	[X01457]	(H-1PV)
00.050.1.01.012.	*Kilham rat virus*		(KRV)
00.050.1.01.012.	(Rat virus)		
00.050.1.01.013.	*Lapine parvovirus*		(LPV)
00.050.1.01.014.	*LUIII virus*	[M81888]	(LUIIIV)
00.050.1.01.016.	*Mice minute virus*	[J02275]	(MMV)
00.050.1.01.015.	*Mink enteritis virus*		(MEV)
00.050.1.01.021.	*Mouse parvovirus 1*	[U12469]	(MPV)
00.050.1.01.017.	*Porcine parvovirus*	[U44978]	(PPV)
00.050.1.01.018.	*Raccoon parvovirus*	[M24005]	(RPV)
00.050.1.01.019.	*RT parvovirus*		(RTPV)
00.050.1.01.020.	*Tumor virus X*		(TVX)

Tentative Species in the Genus

00.050.1.81.023.	Rheumatoid arthritis virus	(RAV-1)

References

Medline References. The following references are cited in the *Seventh ICTV Report*.

Data Sources and Contributions

The description has been compiled from data presented in the *Seventh ICTV Report* by Berns KI, Bergoin M, Bloom M, Lederman M, Muzyczka N, Siegl G, Tal J, Tattersall P.

Images

Taxon images: • EM by Cornelia Büchen-Osmond. • EM from Stewart McNulty, Queens University, Belfast.

A-3. MAD-K87 VIRUS

Also known as Adenoviridae or Mastadenovirus

Classification

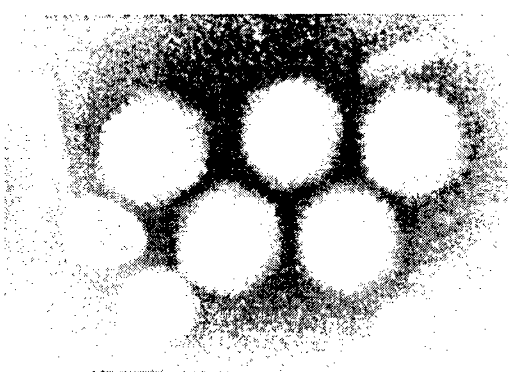
This is a description of a vertebrate virus at the genus level.
ICTVdB *Virus Code*: 00.001.0.01. *Virus accession number*: 001001GE. Former *Virus Code*: 01.0.1.;*former accession number*: 01010000.
NCBI TaxID: [10509] .

Name, Synonyms and Lineage

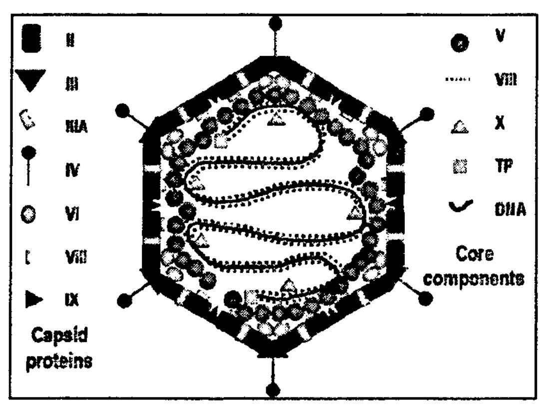
Virus is assigned to the family 00.001. *Adenoviridae*

Morphology

Virions have a simple construction and consist of a capsid, fibers, a core, and associated proteins. Virions are not enveloped. Capsid is round and exhibits icosahedral symmetry. The capsid is isometric and has a diameter of 70–90 nm. Capsids appear hexagonal in outline. The capsid surface structure reveals a regular pattern with distinctive features. The capsomer arrangement is clearly visible. The capsid consists of 252 capsomers. Surface projections are often lost during preparation, or distinct filaments protruding from the 12 vertices.
Capsids all have the same appearance, or can be penetrated by stain and some appear dark in the center; virus may occur together with a dependent virus.



H. Ackermann



Nucleic Acid

The genome is not segmented and consists of a single molecule of linear double-stranded DNA. The complete genome is 30000–36000 nucleotides long. The genome has a guanine + cytosine content of very variable and ranges between 48–61 %. The genome has terminally redundant sequences. The terminally redundant sequences have inverted terminal repetitions (ITR). The genome has a virus coded terminal protein. The 3'–terminus sequences are conserved nucleotide sequences and are 50–200 nucleotides in length.

Proteins

The viral genome encodes structural and non-structural proteins.

Non-Structural Proteins: The virus codes for a DNA-dependent DNA polymerase. In addition to the polymerase, the virus codes for enzymes such as replicase.

Lipids

Lipids are not reported.

Transcription: Virus transcription is temporally regulated. The viral genome is transcribed by host cell enzymes.

During the early stage, the viral genome is transcribed by cellular RNA polymerase II; during the late stage by cellular RNA polymerase III.

Translation: Virions may provide helper functions to dependent virus during replication. Virion acts as helper for a satellite virus.

Biological Properties

Natural Host Range

Virus infects during its life cycle a single type of vertebrate host. Viral hosts belong to the Domain Eucarya.

Domain Eucarya

Kingdom Animalia.

Kingdom Animalia

Phylum Chordata.

Phylum Chordata

Subphylum Vertebrata; Class Mammalia.

Class Mammalia

Order Scandentia, or Primates, or Carnivora, or Perissodactyla, or Artiodactyla, or Rodentia;
Family **Hominidae**; virus infects *Homo sapiens* (human);
Family **Suidae**, or **Bovidae**:.

Taxonomic Structure of the Genus

Type species 00.001.0.01.001. *Human adenovirus C.*

Specific Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, or subspecies are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

00.001.0.01.014.	*Murine adenovirus A*		(MAdV-A)
00.001.0.01.014.00.701.	Murine adenovirus 1	[U95843]	(MAdV-1)

Data Sources and Contributions

The description has been compiled from data presented in the *Seventh ICTV Report* by Benkö, M., Harrach, B., and Russell, W.C. Presented data have been collated for ICTVdB by C. Büchen-Osmond, Oct 2001.

A-4. MURINE HEPATITIS VIRUS (MHV) OR *CORONAVIRIDAE*

Coronaviridae

Table of Contents

Taxonomy

Virus Code. 19. *Virus Accession number* 19000000.

Virus infects vertebrates.

Description is on taxonomic level of family. Virus belongs to the order *Nidovirales* (VC 03.).

Properties of Virion

Morphology

Virions enveloped; slightly pleomorphic; spherical, or kidney-shaped, or rod-shaped; 120-160 nm in diameter, or 120-140 nm in diameter. Surface projections of envelope distinct; club-shaped; spaced widely apart and dispersed evenly over the entire surface. Nucleocapsids rod-shaped (straight or bend); 9 nm in diameter, or 11-13 nm in diameter. Symmetry helical (or tubular).

Physicochemical an Physical Properties

Molecular mass (Mr) of virion 400 x 106. Buoyant density 1.23-1.24 g cm-3 in CsCl; 1.15-1.19 g cm-3 in sucrose. Sedimentation coefficient 300-500 *S*. Under *in vitro* conditions virions some viruses stable; in acid environment (pH 3); virions relatively stable; in presence of Mg++ (heat inactivation of MHV reduced). Virions sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, and oxidizing agents.

Nucleic Acid

Virions contain one molecule of linear positive-sense single stranded RNA.

Total genome length is 20000-33000 nt. The 5' end of the genome has a cap. 3' end has a poly (A) tract. Sub-genomic *m*RNA found in infected cells.

Proteins

Five structural virion proteins found. Protein size 180000-220000 Da, or 200000 Da. Surface glycoprotein (or spike, S). The S protein is responsible for attachment to cells, hemagglutination and membrane fusion. It has a carboxy-terminal half with a coiled-coil structure. Protein size of 2nd largest 30000-35000 Da, or 27000 Da. Integral membrane protein (M), which spans the virus envelope three times with only 10% protruding at the virion surface. Protein size of 3rd largest 50000-60000 Da, or 19000 Da. Nucleocapsid protein (N). Protein size of 4th largest 65000 Da. Hemagglutinine-esterase protein (HE), which forms short surface projections, and can have receptor binding, hemagglutination and receptor destroying activities. Protein size of 5th largest 10000-12000 Da. Tentatively named small membrane protein (sM) detected in avian infectious bronchitis virus (IBV) and porcine transmissible gastroenteritis virus (TGEV). Virion structural proteins are glycosylated. Are the following: the large surface protein (S).

Genome Organization and Replication

Genomic nucleic acid infectious.

Genome replicates in cytoplasm.

Cytopathology. Virions found mature in cytoplasm.

Antigenic Properties

Serological Relationships

Virus elicits distinct antigen determinants on envelope and spikes that are corresponding to each of the major structural glycoproteins. That are corresponding to each of the major virion proteins spike (S), HE, M, and N. Antigenic specificity of virion can be determined by neutralization tests (S and HE), or complement fixation tests (M). Protective immunity is induced in form of complement independent neutralizing antibodies.

Biological Properties

Symptoms and Host Range

Host of virus belongs to the Domain Eucarya. Host of virus belongs to the Kingdom Animalia. Phylum Chordata. Subphylum Vertebrata. Class Mammalia and Aves. Order Primates, Carnivora, Perissodactyla, Artiodactyla, Rodentia, and Lagomorphia.

Transmission

Transmitted by means not involving a vector.

Geographic Distribution

Probably distributed world-wide.

Taxonomic Structure

Taxonomic structure of the family.
Genus *Coronavirus* (VC 19.0.1.) and
Genus *Torovirus* (VC 19.0.2.).

References

The following references are cited in the *Sixth ICTV Report*: rf_coron.htm.

Data sources and contributors.

The above description has been compiled from the data presented in the *Sixth ICTV Report* by Cavanagh D, Brain DA, Brinton MA, Enjuanes L, Holmes KV, Horzinek MC, Lai MMC, Laude H, Plagemann PGW, Siddell SG, Spaan WJM, Taguchi F, Talbot PJ.

Related Databases

Taxon images: corona2.gif

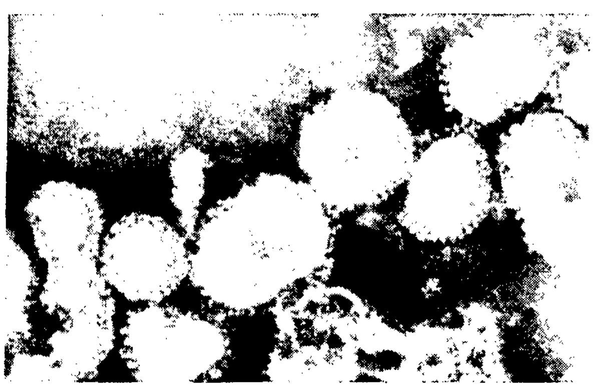
Taxonomy

Approved acronym: MHV.
Virus infects vertebrates.
Description is on taxonomic level of species. Virus is an approved species in the genus. Virus belongs to the genus *Coronavirus* (VC 19.0.1.); family *Coronaviridae* (VC 19.); order *Nidovirales* (VC 03.).

Properties of Virion

Morphology

Virions enveloped; slightly pleomorphic; spherical; (60-)120-160(-200) nm in diameter. Surface projections of envelope distinct; club-shaped (and 12-24 nm in length); spaced widely apart and dispersed evenly over the entire surface. Nucleocapsids filamentous; 9-13 nm in diameter. Symmetry helical.



Nucleic Acid

Virions contain one molecule of linear positive-sense single stranded RNA.
Total genome length is 33000 nt.

Genome Organization and Replication

Genomic nucleic acid infectious.

Data sources and contributors.

The above description has been compiled from the data presented in the *Sixth ICTV Report* by Cavanagh D, Brain DA, Brinton MA, Enjuanes L, Holmes KV, Horzinek MC, Lai MMC, Laude H, Plagemann PGW, Siddell SG, Spaan WJM, Taguchi F, Talbot PJ.

Blank

A-5. MVM PARVOVIRUS

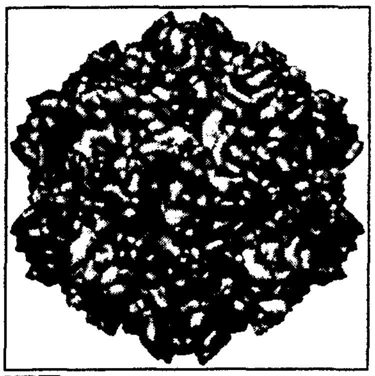
Mice minute virus

Name, Synonyms and Lineage

Alternative name: *Murine minute mice virus*. Synonym(s): *Minute mice virus*. Virus is the type species. Virus is assigned to the genus 00.050.1.01. *Parvovirus*; subfamily 00.050.1. *Parvovirinae*; family 00.050. *Parvoviridae.*

Morphology

Virions have a simple construction and consist of a capsid. Virions are not enveloped. Capsid/nucleocapsid is round and exhibits icosahedral symmetry. The nucleocapsid is isometric and has a diameter of 20-26 nm. Capsids appear round. The capsid consists of 60 capsomers (each a quadrilateral 'kite-shaped' wedge). Surface projections are small and surface appears rough and distinct spikes.



VIPER

Nucleic Acid

The genome is not segmented and consists of a single molecule of linear negative-sense, or negative-sense and positive-sense (up to 50% in some members) single-stranded DNA. The complete genome is 5000 nucleotides long. Genome has been fully sequenced and has the accession number [J02275]. The genome has terminally redundant sequences. The genome sequence is repeated at both ends. The 5'-terminal sequence has palindromic repeats, forming a hairpin structure. Terminal repeats at the 5'-end are 200-242 nucleotides long. The 3'-terminus has conserved nucleotide sequences; { } has 115-116 nucleotides in length; sequence has hairpin structure. Populations of mature viruses contain particles with equivalent numbers of positive and negative sense ssDNA . Upon extraction, the complementary DNA strands usually form dsDNA.

Proteins

The viral genome encodes structural proteins.

Lipids

Lipids are absent.

Translation: Virions are associated with helper virus, but independent from its functions during replication.

Biological Properties

Natural Host Range

Virus infects during its life cycle a single type of vertebrate host. Viral hosts belong to the Domain Eucarya.

Domain Eucarya

Kingdom Animalia.

Kingdom Animalia

Phylum Chordata.

Phylum Chordata

Subphylum Vertebrata; Class Mammalia.

Class Mammalia

Order Rodentia.

Data Sources and Contributions

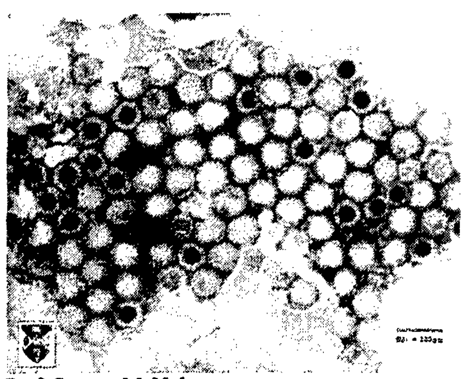
The description has been compiled from data presented in the *Seventh ICTV Report* by Berns KI, Bergoin M, Bloom M, Lederman M, Muzyczka N, Siegl G, Tal J, Tattersall P.

A-6. REO-3 REOVIRUS

Reovirus-Orthoreovirus

Taxonomic Structure
Taxonomic structure of the genus.
Type species: 60.0.1.1.001 reovirus 3.
Species: (*Serogroups*) 60.0.1.1. Mammalian orthoreoviruses; 60.0.1.2. Avian orthoreoviruses.

Morphology
Virions not enveloped. Virions one type of particle only. Nucleocapsids isometric. Capsid shell of virion composed of two layers. Shell of virion all usually present, or outer often lost. Nucleocapsids with obvious regular surface structure; 80-82 nm in diameter. Symmetry icosahedral. Nucleocapsids appear to be round. Surface capsomer arrangement obvious. Surface projections of nucleocapsid not present. Core isometric with a diameter of 60 nm. The diameter of the central compartment where the dsRNA genome is located is 49 nm. Core particles have projections located at each of the 12 capsid vertices. These extend almost to the surface of the virion. Incomplete virus particles often present; they are empty capsids, or incompletely assembled capsids. Virions only of one kind.


Prof. Stewart McNulty

Nucleic Acid
Virions contain 15-20 % nucleic acid. Genome consists of a monomer. Virions contain 10 segments of linear double stranded RNA.
Total genome length is 23700 nt. The largest segment L1 3800-3900 nt; of second largest L2 3800-3900 nt; of third L3 3800-3900 nt; of fourth M1 2200-2300 nt; of fifth M2 2200-2300 nt; of sixth M3 2200-2300 nt; of seventh S1 1200-1400 nt; of eighth S2 1200-1400 nt; of ninth S3 1200-1400 nt; of tenth S4 1200-1400 nt. The 5' end of the genome has a cap (on the positive strand of each duplex, the negative strands have a phosphorylated terminus). Cap sequence m7G5ppp5'GmpNp. 3' end has no poly (A) tract. Encapsulated nucleic acid solely genomic. Genome found in one type of particle only. Each virion contains a single copy of genome; full length copy, or shorter copies (that is defective particles may lack particular dsRNA species, or contain abnormal dsRNA sequences).
Proteins
Virions contain 80-85 % protein.

Data sources and contributors.

The above description has been compiled from the data presented in the *Sixth ICTV Report* by Holmes IH, Boccardo G, Estes MK, Furuichi MK, Hoshino Y, Joklik WK, McCrae M, Mertens PPC, Milne RG, Samal KSK, Shikata E, Winton JR, Uyeda I, and Nuss DL.